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(54) Title: <b>METHOD AND REAGENT FOR TREATMENT OF ARTHRITIC CONDITIONS, INDUCTION OF GRAFT TOLERANCE AND REVERSAL OF IMMUNE RESPONSES</b>			
(57) Abstract			
<p>An enzymatic nucleic acid molecule which cleaves RNA associated with development or maintenance of an arthritic condition, induction of graft tolerance or reversal of an immune response. In particular, the ribozyme sequences are directed to an mRNA encoding B7-1, B7-2, B7-3, CD40 and/or stromelysin. Also provided are ribozymes where the uracil in positions 4 and/or 7 are substituted, as well as methods for the synthesis of 2'-alkylnucleotides, 2'-O-alkylthioalkyl, or 2'-alkylthioalkylnucleotides. The application further describes a method for diprotection of RNA with aqueous ethylamine, a method for synthesis of a basic ribonucleoside mimetics, and transcription units comprising an RNA polymerase II promoter, a U6 small nuclear promoter, or an adenovirus VA1 promoter system.</p>			

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METHOD AND REAGENT FOR TREATMENT OF ARTHRITIC  
CONDITIONS, INDUCTION OF GRAFT TOLERANCE AND  
REVERSAL OF IMMUNE RESPONSES

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Background of the Invention

The following is a discussion of relevant art, none of which is admitted to be prior art to the present invention.

10 In one aspect, this invention relates to methods for inhibition of osteoarthritis, in particular, inhibition of genetic expression which leads to a reduction or elimination of extracellular matrix digestion by matrix metalloproteinases.

15 There are several types of arthritis, with osteoarthritis and rheumatoid arthritis being predominant. Osteoarthritis is a slowly progressive disease characterized by degeneration of articular cartilage with proliferation and remodeling of subchondral bone. It presents with a clinical picture of pain, deformity, and loss of joint motion. Rheumatoid arthritis is a chronic systemic inflammatory disease. Rheumatoid arthritis may be mild and relapsing or severe and progressive, leading to joint deformity and incapacitation.

20 Arthritis is the major contributor to functional impairment among the older population. It is the major cause of disability and accounts for a large proportion of the hospitalizations and health care expenditures of the elderly. Arthritis is estimated to be the principal cause of total incapacitation for about one million persons aged 55 and older and is thought to be an important contributing cause for about one million more.

25 Estimating the incidence of osteoarthritis is difficult for several reasons. First, osteoarthritis is diagnosed objectively on the basis of reading radiographs, but many people with radiologic evidence of disease have no obvious symptoms. Second, the estimates of prevalence are based upon clinical evaluations because radiographic data is not available for all afflicted joints. In the NHANESI survey of 1989, data were based upon a thorough musculoskeletal evaluation during which any abnormalities of the spine, knee,

30

hips, and peripheral joints were noted as well as other specific diagnoses. Based on these observations, 12% of the US population between 25 and 74 years of age have osteoarthritis.

It is generally agreed that rheumatoid arthritis has a world-wide distribution and affects all racial and ethnic groups. The exact prevalence in the US is unknown but has been estimated to range between 0.5% and 1.5%. Rheumatoid arthritis occurs at all age levels and generally increases in prevalence with advancing age. It is 2-3 times more prevalent in women than in men and peak incidence occurs between 40-60 years of age. In addition to immunological factors, environmental, occupational and psychosocial factors have been studied for potential etiologic roles in the disease.

The extracellular matrix of multicellular organisms plays an important role in the formation and maintenance of tissues. The meshwork of the extracellular matrix is deposited by resident cells and provides a framework for cell adhesion and migration, as well as a permeability barrier in cell-cell communication. Connective tissue turnover during normal growth and development or under pathological conditions is thought to be mediated by a family of neutral metalloproteinases, which are zinc-containing enzymes that require calcium for full activity. The regulation of metalloproteinase expression is cell-type specific and may vary among species.

The best characterized of the matrix metalloproteinases, interstitial collagenase (MMP-1), is specific for collagen types I, II, and III. MMP-1 cleaves all three chains of the triple helix at a single point initiating sequential breakdown of the interstitial collagens. Interstitial collagenase activity has been observed in rheumatoid synovial cells as well as in the synovial fluid of patients with inflammatory arthritis. Gelatinases (MMP-2) represent a subgroup of the metalloproteinases consisting of two distinct gene products; a 70 kDa gelatinase expressed by most connective tissue cells, and a 92 kDa gelatinase expressed by inflammatory phagocytes and tumor cells. The larger enzyme is expressed by macrophages, SV-40 transformed fibroblasts, and neutrophils. The smaller enzyme is secreted by H-ras transformed bronchial epithelial cells and tumor cells, as well as normal human skin fibroblasts. These enzymes degrade gelatin (denatured collagen) as well as native



collagen type XI. Stromelysin (MMP-3) has a wide spectrum of action on molecules composing the extracellular matrix. It digests proteoglycans, fibronectin, laminin, type IV and IX collagens and gelatin, and can remove the N-terminal propeptide region from procollagen, thus activating the collagenase. It has been found in human cartilage extracts, rheumatoid synovial cells, and in the synovium and chondrocytes of joints in rats with collagen-induced arthritis.

Both osteoarthritis and rheumatoid arthritis are treated mainly with compounds that inhibit cytokine or growth-factor induced synthesis of the matrix metalloproteinases which are involved in the extracellular matrix destruction observed in these diseases. Current clinical treatments rely upon dexamethasone and retinoid compounds, which are potent suppressors of a variety of metalloproteinases. The global effects of dexamethasone and retinoid treatment upon gene expression in treated cells make the development of alternative therapies desirable, especially for long term treatments. Recently, it was shown that gamma-interferon suppressed lipopolysaccharide induced collagenase and stromelysin production in cultured macrophages. Also, tissue growth factor- $\beta$  (TGF- $\beta$ ) has been shown to block epidermal growth factor (EGF) induction of stromelysin synthesis in vitro. Experimental protocols involving gene therapy approaches include the controlled expression of the metalloproteinase inhibitors TIMP-1 and TIMP-2. Of the latter three approaches, only  $\gamma$ -interferon treatment is currently feasible in a clinical application.

Sullivan and Draper, International PCT Publication No. WO 94/02595 and Draper *et al.*, International PCT Publication No. WO 95/13380 disclose the use of ribozymes to treat arthritis.

In a second aspect, the invention relates to methods for the induction of graft tolerance, treatment of autoimmune diseases, inflammatory disorders and allergies in particular, by inhibition of B7-1, B7-2, B7-3 and CD40.

An adaptive immune response requires activation, clonal expansion, and differentiation of a class of cells termed T lymphocytes (T cells). T cell activation is a multi-step process requiring several signalling events between

the T cell and an antigen presenting cell. The ensuing discussion details signals that are exchanged between T cells and antigen presenting B cells. Similar pathways are thought to occur between T cells and other antigen presenting cells such as monocytes or follicular dendritic cells.

- 5        T cell activation is initiated when the T-cell receptor (TCR) binds to a specific antigen that is associated with the MHC proteins on the surface of an antigen presenting cell. This primary stimulus activates the T cell and induces expression of CD40 ligand (CD40L) on the surface of the T cell. CD40L then interacts with its cognate receptor, CD40, which is constitutively expressed on
- 10    the surface of B cells; CD40 transduces the signal leading to B cell activation. B cell activations result in the expression of B7-1, B7-2 and/or B7-3, which in turn interacts with constitutively expressed CD28 on the surface of T cells. The interaction generates a secondary co-stimulatory signal that is required to fully activate the T cell. Complete T cell activation via the T cell receptor and CD28
- 15    leads to cytokine secretion, clonal expansion, and differentiation. If the T cell receptor is engaged, absence of this secondary co-stimulus mediated by CD28, then the T cell is inactivated, either by clonal anergy (non-responsiveness or reduced reactivity of the immune system to specific antigen(s)) or clonal deletion (Jenkins et al., 1987 *Proc. Natl. Acad. Sci. USA*
- 20    84, 5409). Thus, engagement of the TCR without a concomitant costimulatory signal results in a state of tolerance toward the specific antigen recognized by the T cell. This co-stimulatory signal can be mediated by the binding of B7-1 or B7-2 or B7-3, present on activated antigen-presenting cells, to CD28, a receptor that is constitutively expressed on the surface of the T cell
- 25    (Marshall et al., 1993 *J Clin Immun* 13, 165-174; Linsley, et al., 1991 *J Exp Med* 173, 721; Koulouva et al., 1991 *J Exp Med* 173, 759; Harding et al., 1992 *Nature* 356, 607).

- Several homologs of B7 (now known as B7-1; Cohen, 1993 *Science* 262, 844) are expressed in activated B cells (Freeman et al., 1993 *Science*
- 30    262, 907; Lenschow et al., 1993 *Proc Natl Acad Sci USA* 90, 11054; Azuma et al., 1993 *Nature* 366, 76; Hathcock et al., 1993 *Science* 262, 905; Freeman et al., 1993 *Science* 262, 909). B7-1 and B7-3 are only expressed on the surface of a subset of B cells after 48 hours of contact with T cells. In contrast, B7-2 mRNA is constitutively expressed by unstimulated B cells and increases 4-fold

within 4 hours of activation (Freeman et al., 1993 *Science* 262, 909; Boussiotis et al., 1993 *Proc Natl Acad Sci USA* 90, 11059). Since T cells commit to either the anergy or the activation pathway within 12-24 hours of the initial TCR signal, it is thought that B7-2 is the molecule responsible for the primary  
5 costimulatory signal. B7-1 and B7-3 may provide a subsequent signal necessary for clonal expansion. Antibodies to B7-2 completely block T cell proliferation in a mixed lymphocyte reaction (Azuma et al., 1993 *supra*), supporting the central role of B7-2 in T cell activation. These experiments indicate that inhibition of B7-2 expression (for example with a ribozyme) would  
10 likely induce anergy. Similarly, inhibition of CD40 expression by a ribozyme would prevent B7-2 upregulation and could induce tolerance to specific antigens.

B7 (B7-1) is a 60 KD modified trans-membrane glycoprotein usually present on the surface of antigen presenting cells (APC). B7 has two ligands—  
15 CD28 and CTLA4. Interaction of B7-1 with CD28 and/or CTLA4 causes activation of T cell responses (Janeway and Bottomly, 1994 *Cell* 76, 275).

B7-2 is a 70 KD (34 KD unmodified) trans-membrane glycoprotein found on the surface of APCs. B7-2 encodes a 323 amino-acid protein which is 26 % identical to human B7-1 protein. Like B7-1, CD28 and CTLA4 are  
20 selectively bound by B7-2. B7-2, unlike B7-1, is expressed on the surface of unstimulated B cells (Freeman et al., 1993 *supra*).

CD40 is a 45-50 KD surface glycoprotein found on the surface of late pre-B cells in bone marrow, mature B cells, bone marrow-derived dendritic cells and follicular dendritic cells (Clark and Ledbetter, 1994 *Nature* 367, 425).

25 Successful organ transplantation currently requires suppression of the recipient's immune system in order to prevent graft rejection and maintain good graft function. The available therapies, including cyclosporin A, FK506 and various monoclonal antibodies, all have serious side effects (Caine, 1992 *Transplantation Proceedings* 24, 1260; Fuleihan et al., 1994 *J. Clin. Invest.* 93, 1315; Van Gool et al., 1994 *Blood* 83, 176). In addition, existing therapies  
30 result in general immune suppression, leaving the patient susceptible to a variety of opportunistic infections. The ability to induce a state of long-term,

antigen-specific tolerance to the donor tissue would revolutionize the field of organ and tissue transplantation. Since organ graft rejection is mediated by T cell effector function, the goal is to block specifically the activation of the subset of T cells that recognize donor antigens. A limitation in the field of  
5 transplantation is the supply of donor organs (Nowak 1994 *Science* 266, 1148). The ability to induce donor-specific tolerance would substantially increase the chances of successful allografts, xenografts, thereby greatly increasing the donor pool.

Such transplantation includes grafting of tissues and/or organ i.e.,  
10 implantation or transplantation of tissue and/or organs, from the body of an individual to a different place within the same or different individual. Transplantation also involve grafting of tissues and/or organs from one area of the body to another. Transplantation of tissues and/or organs between genetically dissimilar animals of the same species is termed as allogeneic  
15 transplantation. Transplantation of animal organs into humans is termed xenotransplants (for a review see Nowak; 1994 *Science* 266, 1148).

One therapy currently being developed that has similar potential to induce antigen-specific tolerance is treatment with a CTLA4-Ig fusion protein. "CTLA4" is a homologue of CD28 that binds B7-1 and B7-2 with high affinity.  
20 The engineered, soluble fusion protein, CTLA4-Ig, binds B7-1, thereby blocking its interaction with CD28. The results of CTLA4-Ig treatment in animal studies are mixed. CTLA4-Ig treatment significantly enhanced survival rates and ameliorated the symptoms of graft-versus host disease in a murine bone marrow transplant model (Blazer et al., 1994 *Blood* 83, 3815). CTLA4-Ig  
25 induced long-term (>110 days) donor-specific tolerance in pancreatic islet xenografts (Lenschow et al., 1992 *Science* 257, 789). Conversely, in another study CTLA4-Ig treatment delayed but did not ultimately prevent cardiac allograft rejection (Turka, et al., 1992 *Proc Natl Acad Sci U S A* 89, 11102). Mice immunized with sheep erythrocytes in the presence of CTLA4-Ig failed to  
30 mount a primary immune response (Linsley, et al., 1992 *Science* 257, 792). A secondary immunization did elicit some response, however, indicating incomplete tolerance. Interestingly, identical results were obtained when CTLA4-Ig was administered 2 days after primary immunization, leading the authors to conclude that CTLA4-Ig blocked amplification rather than initiation

of the immune response. Since CTLA4-Ig has been shown to dissociate more rapidly from B7-2 compared with B7-1, this may explain the failure to induce long term tolerance in this model (Linsley et al., 1994 *Immunity* 1, 793).

CTLA4:Ig has recently been shown to ameliorate symptoms of spontaneous autoimmune disease in lupus-prone mice (Finck et al., 1994 *Science* 265, 1225).

Linsley et al., WO 92/00092 describe B7 antigen as a ligand for CD28 receptor on T cells. The application states that—

10 "The B7 antigen, or its fragments or derivatives are reacted with CD28 positive T cells to regulate T cell interactions with other cells..... B7 antigen or CD28 receptor may be used to inhibit interaction of cells associated with these molecules, thereby regulating T cell responses."

De Boer and Conroy, WO 94/01547 describe the use of anti-B7 and anti-CD40 antibodies to treat allograft transplant rejection, graft versus host disease and rheumatoid arthritis. The application states that—

15 "...anti-B7 and anti-CD40 antibodies...can be used to prevent or treat an antibody-mediated or immune system disease in a patient."

Since signalling via CD40 precedes induction of B-7, blocking the CD40-CD40L interaction would also have the potential to produce tolerance. According to one report, simultaneous treatment of mice with antibodies to CD40L and sheep red blood cells produced antigen-specific tolerance for up to 3 weeks following cessation of treatment (Foy et al., 1993 *J Exp Med* 178, 1567). Anti-CD40L also produces antigen specific tolerance in a pancreatic islet transplant model (R. Noelle, personal communication). Targeted inhibition of CD40 expression in B cells in addition to B7 would therefore afford double protection against activation of T cells.

Therapeutic agents used to prevent rejection of a transplanted organ are all cytotoxic compounds or antibodies designed to suppress the cell-mediated immune system. The side effects of these agents are those of immunosuppression and infections. The primary approved agents are azathioprine, corticosteroids, cyclosporine; the antibodies are antilymphocyte or antithymocyte globulins. All of these are given to individuals who have been as closely matched as possible to their donors by both major and minor

histocompatibility typing. Since the principal problem in transplantation is an antigenic mismatch and the resulting need for cytotoxic therapy, any therapeutic improvement which decreases the local immune response without general immunosuppression should capture the transplant market.

- 5        Cyclosporine: At the end of the 1970's and early 1980's the introduction of cyclosporine revolutionized the transplantation field. It is a potent immunosuppressant which can inhibit immunocompetent lymphocytes specifically and reversibly. Its primary mechanism of action appears to be inhibition of the production and release of interleukin-2 by T helper cells. In  
10 addition it also interferes with the release of interleukin-1 by macrophages, as well as proliferation of B lymphocytes. It was approved by the FDA in 1983 and by 1989 was almost universally given to transplant recipients. At first it was believed that the toxicity and side effects from cyclosporine were minimal and it was hailed as a "wonder drug." Numerous side effects have been  
15 progressively cited, including the appearance of lymphomas, especially in the gastrointestinal tract; acute and chronic nephrotoxicity; hypertension; hepatotoxicity; hirsutism; anemia; neurotoxicity; endocrine and neurological complications; and gastrointestinal distress. It is now widely acknowledged that the non-specific side effects of the drug demand caution and close  
20 monitoring of its use. One-year survival rates for cadaver kidney transplants treated with cyclosporine is 80%, much better than the 50-60% rates without the drug. The one-year survival is almost 90% for transplants with related donors and the use of cyclosporine.

- 25        Azathioprine: In addition to cyclosporine, azathioprine is used for transplant patients. Azathioprine is one of the mercaptopurine class of drugs and inhibits nucleic acid synthesis. Patients are maintained indefinitely on daily doses of 1mg/kg or less, with a dosage adjusted in accordance with the white cell count. The drug may cause depression of bone marrow elements and may cause jaundice.

- 30        Corticosteroids: Prednisone, used in almost all transplant recipients, is usually given in association with azathioprine and cyclosporine. The dosage must be regulated carefully so as to prevent complications such as infection, development of cushingoid features, and hypertension. Usually the initial

maintenance prednisone dosage is 0.5 mg/kg/d. This dosage is usually further decreased in the outpatient clinic until maintenance levels of about 10 mg/d for adults are obtained. The exact site of action of corticosteroids on the immune response is not known.

- 5        Antithymoblast or antilymphocyte globulin (ALG) and antithymocyte globulin (ATG): These are important adjunctive immunosuppressants. They are effective, particularly in induction of immunosuppressive therapy and in the treatment of corticosteroid-resistant rejection. Both ALG and ATG can be made by immunizing horses, rabbits, or sheep; the main source is horses.
- 10      Lymphocytes from human peripheral blood, spleen, lymph nodes, or thymus serve as the immunogen.

- Tacrolimus: On April 13, 1994 the Food and Drug Administration approved another drug to help prevent the rejection of organ transplants. The drug, tacrolimus, was approved only for use in liver transplant patients. An
- 15      alternative to cyclosporine, the macrolide immunosuppressant tacrolimus is a powerful and selective anti-T-lymphocyte agent that was discovered in 1984. Tacrolimus, isolated from the fungus *Streptomyces tsukubaensis*, possesses immunodepressant properties similar to but more potent than cyclosporine. It inhibits both cell-mediated and humoral immune responses. Like
- 20      cyclosporine, tacrolimus demonstrates considerable interindividual variation in its pharmacokinetic profile. Most clinical studies with tacrolimus have neither been published in their entirety nor subjected to extensive peer review; there is also a paucity of published randomized investigations of tacrolimus vs. cyclosporine, particularly in renal transplantation. Despite these drawbacks,
- 25      tacrolimus has shown notable efficacy as a rescue or primary immunosuppressant therapy when combined with corticosteroids. The potential for reductional withdrawal of corticosteroid therapy with tacrolimus appears to be a distinct advantage compared with the cyclosporine. This benefit may be enhanced by reduced incidence of infectious complications,
- 30      hypertension and hypercholesterolemia reported by some investigators. In other respects, the tolerability profile of tacrolimus appears to be broadly similar to that of cyclosporine.

In addition to induction of graft tolerance, T cell anergy can be used to reverse autoimmune diseases. Autoimmune diseases represent a broad category of conditions. A few examples include insulin-dependent diabetes mellitus (IDDM), multiple sclerosis (MS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), myasthenia gravis (MG), and psoriasis. These seemingly disparate diseases all share the common feature of inappropriate immune response to specific self-antigens. Finck et al. *supra* have reported that CTLA4Ig treatment of mice blocked auto-antibody production in a mice model of SLE. In fact, this effect was observed even when the CTLA4Ig treatment was initiated during the advanced stages of the disease, suggesting that the autoimmune response was a reversible process.

Chappel, WO 94/11011 describes methods to treat autoimmune diseases by inducing tolerance to cells, tissues and organs. The application states that—

15 "Cells genetically engineered with DNA encoding a plurality of antigens of a cell, tissue, or organ to which tolerance is to be induced. The cells are free of co-stimulatory antigens, such as B7 antigen. Such cells induce T-cell anergy against the proteins encoded by the DNA, and may be administered to a patient in order to prevent the onset of or to treat an autoimmune disease, or to induce tolerance to a tissue or organ prior to transplantation."

20 Allergic reactions represent an immediate hypersensitivity response to environmental antigens, typically mediated by IgE antibodies. The ability to induce antigen-specific tolerance provides a powerful avenue to alleviate allergies by exposure to the antigen in conjunction with down-regulation of B7-1, B7-2, B7-3 or CD40.

25 The specific roles of B7-1, B7-2 and B7-3 in T cell activation remains to be determined. Some studies suggest that their functions are essentially redundant (Hathcock et al 1994 *J Exp. Med.* 180, 631), or that the differences observed in the kinetics of expression might simply indicate that B7-2 is important in the initiation of the co-stimulatory signal, while B7-1 plays a role in the amplification of that signal. Other studies point to more specific functions. For example, Kuchroo et al., 1995 *Cell* 80, 707, have reported that blocking B7-1 expression may favor a Th2 response, while blocking B7-2 expression favors a Th1 response. These two helper T cell subpopulations play distinct roles in the immune response and inflammatory disease. Th1 cells are



strongly correlated with auto-immune disease. Allergic responses are typically triggered by Th2 responses. Therefore, the decision to target B7-1, B7-2, CD40 or a combination of the above will depend on the particular disease application.

5

### Summary of the Invention

Applicant notes that the inhibition of collagenase and stromelysin production in the synovial membrane of joints can be accomplished using ribozymes and antisense molecules. Ribozyme treatment can be a partner to  
10 current treatments which primarily target immune cells reacting to pre-existing tissue damage. Early ribozyme or antisense treatment which reduces the collagenase or stromelysin-induced damage can be followed by treatment with the anti-inflammatories or retinoids, if necessary. In this manner, expression of the proteinases can be controlled at both transcriptional and  
15 translational levels. Ribozyme or antisense treatment can be given to patients expressing radiological signs of osteoarthritis prior to the expression of clinical symptoms. Ribozyme or antisense treatment can impact the expression of stromelysin without introducing the non-specific effects upon gene expression which accompany treatment with the retinoids and dexamethasone. The  
20 ability of stromelysin to activate procollagenase indicates that a ribozyme or antisense molecule which reduces stromelysin expression can also be used in the treatment of both osteoarthritis (which is primarily a stromelysin-associated pathology) and rheumatoid arthritis (which is primarily related to enhanced collagenase activity).

25 While a number of cytokines and growth factors induce metalloproteinase activities during wound healing and tissue injury of a pre-osteoarthritic condition, these molecules are not preferred targets for therapeutic intervention. Primary emphasis is placed upon inhibiting the molecules which are responsible for the disruption of the extracellular matrix,  
30 because most people will be presenting radiologic or clinical symptoms prior to treatment. The most versatile of the metalloproteinases (the molecule which can do the most structural damage to the extracellular matrix, if not regulated)

is stromelysin. Additionally, this molecule can activate procollagenase, which in turn causes further damage to the collagen backbone of the extracellular matrix. Under normal conditions, the conversion of prostromelysin to active stromelysin is regulated by the presence of inhibitors called TIMPs (tissue inhibitors of MMP). Because the level of TIMP in synovial cells exceeds the level of prostromelysin and stromelysin activity is generally absent from the synovial fluid associated with non-arthritic tissues, the toxic effects of inhibiting stromelysin activity in non-target cells should be negligible.

Thus, the invention features use of specific ribozyme molecules to treat or prevent arthritis, particularly osteoarthritis, by inhibiting the synthesis of the prostromelysin molecule in synovial cells, or by inhibition of other matrix metalloproteinases discussed above. Cleavage of targeted mRNAs (stromelysin mRNAs, including stromelysin 1, 2, and 3, and collagenase) expressed in macrophages, neutrophils and synovial cells represses the synthesis of the zymogen form of stromelysin, prostromelysin.

Ribozymes are RNA molecules having an enzymatic activity which is able to repeatedly cleave other separate RNA molecules in a nucleotide base sequence specific manner. It is said that such enzymatic RNA molecules can be targeted to virtually any RNA transcript and efficient cleavage has been achieved *in vitro*. Kim et al., 84 Proc. Nat. Acad. of Sci. USA 8788, 1987; Haseloff and Gerlach, 334 Nature 585, 1988; Cech, 260 JAMA 3030, 1988; and Jefferies et al., 17 Nucleic Acid Research 1371, 1989.

Six basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds *in trans* (and thus can cleave other RNA molecules) under physiological conditions. Table I summarizes some of the characteristics of these ribozymes. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct

synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

By "enzymatic RNA molecule" it is meant an RNA molecule which has  
5 complementarity in a substrate binding region to a specified mRNA target, and also has an enzymatic activity which is active to specifically cleave that mRNA. That is, the enzymatic RNA molecule is able to intermolecularly cleave mRNA and thereby inactivate a target mRNA molecule. This complementarity  
10 functions to allow sufficient hybridization of the enzymatic RNA molecule to the target RNA to allow the cleavage to occur. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention. For *in vivo* treatment, complementarity between 30 and 45 bases is preferred; although lower numbers are also useful.

By "complementary" is meant a nucleotide sequence that can form  
15 hydrogen bond(s) with other nucleotide sequence by either traditional Watson-Crick or other non-traditional types (for example Hoogsteen type) of base-paired interactions.

The enzymatic nature of a ribozyme is advantageous over other technologies, such as antisense technology (where a nucleic acid molecule  
20 simply binds to a nucleic acid target to block its translation) since the concentration of ribozyme necessary to affect a therapeutic treatment is lower than that of an antisense oligonucleotide. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly  
25 specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme. Similar mismatches in antisense molecules do not  
30 prevent their action (Woolf, T. M., et al., 1992, Proc. Natl. Acad. Sci. USA, 89, 7305-7309). Thus, the specificity of action of a ribozyme is greater than that of an antisense oligonucleotide binding the same RNA site.

In preferred embodiments of this invention, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif, but may also be formed in the motif of a hepatitis delta virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or *Neurospora* VS RNA. Examples of such hammerhead motifs are described by Rossi *et al.*, 1992, Aids Research and Human Retroviruses 8, 183, of hairpin motifs by Hampel *et al.*, EPA 0360257, Hampel and Tritz, 1989 Biochemistry 28, 4929, and Hampel *et al.*, 1990 Nucleic Acids Res. 18, 299, and an example of the hepatitis delta virus motif is described by Perrotta and Been, 1992 Biochemistry 31, 16; of the RNaseP motif by Guerrier-Takada *et al.*, 1983 Cell 35, 849, *Neurospora* VS RNA ribozyme motif is described by Collins (Saville and Collins, 1990 Cell 61, 685-696; Saville and Collins, 1991 Proc. Natl. Acad. Sci. USA 88, 8826-8830; Collins and Olive, 1993 Biochemistry 32, 2795-2799) and of the Group I intron by Cech *et al.*, U.S. Patent 4,987,071. These specific motifs are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule.

The invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target stromelysin encoding mRNAs such that specific treatment of a disease or condition can be provided with either one or several enzymatic nucleic acids. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from DNA or RNA vectors that are delivered to specific cells.

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small enzymatic nucleic acid motifs (*e.g.*, of the hammerhead or the hairpin structure) are used for exogenous delivery. The simple structure of these molecules increases the ability of the enzymatic nucleic acid to invade targeted regions of the mRNA structure. However,

these catalytic RNA molecules can also be expressed within cells from eukaryotic promoters (e.g., Scanlon et al., 1991, Proc. Natl. Acad. Sci. USA, 88, 10591-5; Kashani-Sabet et al., 1992 Antisense Res. Dev., 2, 3-15; Dropulic et al., 1992 J. Virol., 66, 1432-41; Weerasinghe et al., 1991 J. Virol., 65, 5531-4; Ojwang et al., 1992 Proc. Natl. Acad. Sci. USA, 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Sarver et al., 1990 Science 247, 1222-1225; Thompson et al., 1995 Nucleic Acids Res., 23, 2259). Those skilled in the art realize that any ribozyme can be expressed in eukaryotic cells from the appropriate DNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Draper et al., PCT WO 93/23569, and Sullivan et al., PCT WO 94/02595; Ohkawa et al., 1992 Nucleic Acids Symp. Ser., 27, 15-6; Taira et al., 1991, Nucleic Acids Res., 19, 5125-30; Ventura et al., 1993 Nucleic Acids Res., 21, 3249-55; Chowrira et al., 1994 J. Biol. Chem., 269, 25856).

15 Ribozyme therapy, due to its exquisite specificity, is particularly well-suited to target mRNA encoding factors that contribute to disease pathology. Thus, ribozymes that cleave stromelysin mRNAs may represent novel therapeutics for the treatment of asthma.

20 Thus, in a first aspect, the invention features ribozymes that inhibit stromelysin production. These chemically or enzymatically synthesized RNA molecules contain substrate binding domains that bind to accessible regions of their target mRNAs. The RNA molecules also contain domains that catalyze the cleavage of RNA. The RNA molecules are preferably ribozymes of the hammerhead or hairpin motif. Upon binding, the ribozymes cleave the target  
25 stromelysin encoding mRNAs, preventing translation and stromelysin protein accumulation. In the absence of the expression of the target gene, a therapeutic effect may be observed.

By "inhibit" is meant that the activity or level of stromelysin encoding mRNAs and protein is reduced below that observed in the absence of the  
30 ribozyme, and preferably is below that level observed in the presence of an inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave that RNA.

Such ribozymes are useful for the prevention of the diseases and conditions discussed above, and any other diseases or conditions that are related to the level of stromelysin activity in a cell or tissue. By "related" is meant that the inhibition of stromelysin mRNAs and thus reduction in the level of stromelysin activity will relieve to some extent the symptoms of the disease or condition.

Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The RNA or RNA complexes can be locally administered to relevant tissues ex vivo, or in vivo through injection, aerosol inhalation, infusion pump or stent, with or without their incorporation in biopolymers. In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables AII, AIII, AIV, AVI, AVIII and AIX. Examples of such ribozymes are shown in Tables AV, AVII, AVIII and AIX. Examples of such ribozymes consist essentially of sequences defined in these Tables.

By "consists essentially of" is meant that the active ribozyme contains an enzymatic center equivalent to those in the examples, and binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage.

In a related aspect the invention features ribozymes that cleave target molecules and inhibit stromelysin activity are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme expressing viral vectors could be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the ribozymes are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of ribozymes. Such vectors might be repeatedly administered as necessary. Once expressed, the ribozymes cleave the target mRNA. Delivery of ribozyme expressing vectors could be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell.

By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

This class of chemicals exhibits a high degree of specificity for cleavage of the intended target mRNA. Consequently, the ribozyme agent will only  
5 affect cells expressing that particular gene, and will not be toxic to normal tissues.

The invention can be used to treat or prevent (prophylactically) osteoarthritis or other pathological conditions which are mediated by metalloproteinase activation. The preferred administration protocol is *in vivo*  
10 administration to reduce the level of stromelysin activity.

Thus, the invention features an enzymatic RNA molecule (or ribozyme) which cleaves mRNA associated with development or maintenance of an arthritic condition, e.g., mRNA encoding stromelysin, and in particular, those mRNA targets disclosed in the accompanying tables, which include both  
15 hammerhead and hairpin target sites. In each case the site is flanked by regions to which appropriate substrate binding arms can be synthesized and an appropriate hammerhead or hairpin motif can be added to provide enzymatic activity, by methods described herein and known in the art. For example, referring to Figure 1, arms I and III are modified to be specific  
20 substrate-binding arms, and arm II remains essentially as shown.

Ribozymes that cleave stromelysin mRNAs represent a novel therapeutic approach to arthritic disorders like osteoarthritis. The invention features use of ribozymes to treat osteoarthritis, e.g., by inhibiting the synthesis of prostromelysin molecule in synovial cells or by the inhibition of matrix  
25 metalloproteinases. Applicant indicates that ribozymes are able to inhibit the secretion of stromelysin and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described that other ribozymes that cleave stromelysin encoding mRNAs may be readily designed and are within the  
30 invention.

In other related aspects, the invention features a mammalian cell which includes an enzymatic RNA molecule as described above. Preferably, the

mammalian cell is a human cell; and the invention features an expression vector which includes nucleic acid encoding an enzymatic RNA molecule described above, located in the vector, e.g., in a manner which allows expression of that enzymatic RNA molecule within a mammalian cell; or a  
5 method for treatment of a disease or condition by administering to a patient an enzymatic RNA molecule as described above.

The invention provides a class of chemical cleaving agents which exhibit a high degree of specificity for the mRNA causative of an arthritic condition. Such enzymatic RNA molecules can be delivered exogenously or  
10 endogenously to infected cells. In the preferred hammerhead motif the small size (less than 40 nucleotides, preferably between 32 and 36 nucleotides in length) of the molecule allows the cost of treatment to be reduced.

The enzymatic RNA molecules of this invention can be used to treat arthritic or prearthritic conditions. Such treatment can also be extended to  
15 other related genes in nonhuman primates. Affected animals can be treated at the time of arthritic risk detection, or in a prophylactic manner. This timing of treatment will reduce the chance of further arthritic damage.

In another aspect, the invention features novel nucleic acid-based techniques [e.g., enzymatic nucleic acid molecules (ribozymes), antisense  
20 nucleic acids, 2-5A antisense chimeras, triplex DNA, antisense nucleic acids containing RNA cleaving chemical groups (Cook et al., U.S. Patent 5,359,051)] and methods for their use to induce graft tolerance, to treat autoimmune diseases such as lupus, rheumatoid arthritis, multiple sclerosis and to treatment of allergies.

25 In a preferred embodiment, the invention features use of one or more of the nucleic acid-based techniques to induce graft tolerance by inhibiting the synthesis of B7-1, B7-2, B7-3 and CD40 proteins.

Those in the art will recognize the other potential targets, for e.g., ICAM-1, VCAM-1,  $\beta$ 1 integrin (VLA4) are also suitable for treatment with the nucleic  
30 acid-based techniques described in the present invention.



By "inhibit" is meant that the activity of B7-1, B7-2, B7-3 and/or CD40 or level of mRNAs encoded by B7-1, B7-2, B7-3 and/or CD40 is reduced below that observed in the absence of the nucleic acid. In one embodiment, inhibition with ribozymes preferably is below that level observed in the presence of an enzymatically inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave that RNA.

By "equivalent" RNA to B7-1, B7-2, B7-3 and/or CD40 is meant to include those naturally occurring RNA molecules associated with graft rejection in various animals, including human, mice, rats, rabbits, primates and pigs.

By "antisense nucleic acid" is meant a non-enzymatic nucleic acid molecule that binds to another RNA (target RNA) by means of RNA-RNA or RNA-DNA or RNA-PNA (protein nucleic acid; Egholm et al., 1993 *Nature* 365, 566) interactions and alters the activity of the target RNA (for a review see Stein and Cheng, 1993 *Science* 261, 1004).

By "2-5A antisense chimera" is meant, an antisense oligonucleotide containing a 5' phosphorylated 2'-5'-linked adenylate residues. These chimeras bind to target RNA in a sequence-specific manner and activate a cellular 2-5A-dependent ribonuclease which in turn cleaves the target RNA (Torrence et al., 1993 *Proc. Natl. Acad. Sci. USA* 90, 1300).

By "triplex DNA" is meant an oligonucleotide that can bind to a double-stranded DNA in a sequence-specific manner to form a triple-strand helix. Triple-helix formation has been shown to inhibit transcription of the targeted gene (Duval-Valentin et al., 1992 *Proc. Natl. Acad. Sci. USA* 89, 504).

By "gene" is meant a nucleic acid that encodes an RNA.

Ribozymes that cleave the specified sites in B7-1, B7-2, B7-3 and/or CD40 mRNAs represent a novel therapeutic approach to induce graft tolerance and treat autoimmune diseases, allergies and other inflammatory conditions. Applicant indicates that ribozymes are able to inhibit the activity of B7-1, B7-2, B7-3 and/or CD40 and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described that other ribozymes that cleave these

sites in B7-1, B7-2, B7-3 and/or CD40 mRNAs may be readily designed and are within the invention.

In a preferred embodiment the invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target mRNAs encoding B7-1, B7-2, B7-3 and/or CD40 proteins such that specific treatment of a disease or condition can be provided with either one or several enzymatic nucleic acids. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from DNA/RNA vectors that are delivered to specific cells.

Such ribozymes are useful for the prevention of the diseases and conditions discussed above, and any other diseases or conditions that are related to the levels of B7-1, B7-2, B7-3 and/or CD40 activity in a cell or tissue. By "related" is meant that the inhibition of B7-1, B7-2, B7-3 and/or CD40 mRNAs and thus reduction in the level respective protein activity will relieve to some extent the symptoms of the disease or condition.

Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues ex vivo, or in vivo through injection, infusion pump or stent, with or without their incorporation in biopolymers. In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables BII, BIV, BVI, BVIII, BX, BXII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX. Examples of such ribozymes are shown in Tables BIII, BV, BVI, BVII, BIX, BXI, BXIII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX. Examples of such ribozymes consist essentially of sequences defined in these Tables.

In another aspect of the invention, ribozymes that cleave target molecules and inhibit B7-1, B7-2, B7-3 and/or CD40 activity are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme expressing viral vectors could be constructed based on, but not limited to, adeno-

associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the ribozymes are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of ribozymes. Such vectors might be  
5 repeatedly administered as necessary. Once expressed, the ribozymes cleave the target mRNA. Delivery of ribozyme expressing vectors could be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that would allow for  
10 introduction into the desired target cell.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

15

#### Description of the Preferred Embodiments

The drawings will first briefly be described.

#### Drawings

Figure 1 is a diagrammatic representation of the hammerhead ribozyme domain known in the art. Stem II can be  $\geq 2$  base-pairs long.

20

Figure 2a is a diagrammatic representation of the hammerhead ribozyme domain known in the art; Figure 2b is a diagrammatic representation of the hammerhead ribozyme as divided by Uhlenbeck (1987, Nature, 327, 596-600) into a substrate and enzyme portion; Figure 2c is a similar diagram showing the hammerhead divided by Haseloff and Gerlach (1988, Nature, 334, 585-  
25 591) into two portions; and Figure 2d is a similar diagram showing the hammerhead divided by Jeffries and Symons (1989, Nucl. Acids. Res., 17, 1371-1371) into two portions.

25

Figure 3 is a diagrammatic representation of the general structure of a hairpin ribozyme. Helix 2 (H2) is provided with a least 4 base pairs (*i.e.*, *n* is  
30 1, 2, 3 or 4) and helix 5 can be optionally provided of length 2 or more bases

(preferably 3 - 20 bases, *i.e.*, *m* is from 1 - 20 or more). Helix 2 and helix 5 may be covalently linked by one or more bases (*i.e.*, *r* is  $\geq 1$  base). Helix 1, 4 or 5 may also be extended by 2 or more base pairs (*e.g.*, 4 - 20 base pairs) to stabilize the ribozyme structure, and preferably is a protein binding site. In each instance, each *N* and *N'* independently is any normal or modified base and each dash represents a potential base-pairing interaction. These nucleotides may be modified at the sugar, base or phosphate. Complete base-pairing is not required in the helices, but is preferred. Helix 1 and 4 can be of any size (*i.e.*, *o* and *p* is each independently from 0 to any number, *e.g.*, 20) as long as some base-pairing is maintained. Essential bases are shown as specific bases in the structure, but those in the art will recognize that one or more may be modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Helix 4 can be formed from two separate molecules, *i.e.*, without a connecting loop. The connecting loop when present may be a ribonucleotide with or without modifications to its base, sugar or phosphate. "*q*" is  $\geq 2$  bases. The connecting loop can also be replaced with a non-nucleotide linker molecule. *H*, refers to bases A, U or C. *Y* refers to pyrimidine bases. "-" refers to a chemical bond.

Figure 4 is a representation of the general structure of the hepatitis delta virus ribozyme domain known in the art.

Figure 5 is a representation of the general structure of the self-cleaving VS RNA ribozyme domain.

Figure 6 is a schematic representation of an RNaseH accessibility assay. Specifically, the left side of Figure 6 is a diagram of complementary DNA oligonucleotides bound to accessible sites on the target RNA. Complementary DNA oligonucleotides are represented by broad lines labeled A, B, and C. Target RNA is represented by the thin, twisted line. The right side of Figure 6 is a schematic of a gel separation of uncut target RNA from a cleaved target RNA. Detection of target RNA is by autoradiography of body-labeled, T7 transcript. The bands common to each lane represent uncleaved target RNA; the bands unique to each lane represent the cleaved products.

Figure 7 shows *in vitro* cleavage of stromelysin mRNA by HH ribozymes.

Figure 8 shows inhibition of stromelysin expression by 21HH ribozyme in HS-27 fibroblast cell line.

Figure 9 shows inhibition of stromelysin expression by 463HH ribozyme  
5 in HS-27 fibroblast cell line.

Figure 10 shows inhibition of stromelysin expression by 1049HH ribozyme in HS-27 fibroblast cell line.

Figure 11 shows inhibition of stromelysin expression by 1366HH ribozyme in HS-27 fibroblast cell line.

10 Figure 12 shows inhibition of stromelysin expression by 1410HH ribozyme in HS-27 fibroblast cell line.

Figure 13 shows inhibition of stromelysin expression by 1489HH ribozyme in HS-27 fibroblast cell line.

15 Figure 14 shows 1049HH ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

Figure 15 shows 1049HH ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

Figure 16 shows 1049HH ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

20 Figure 17 shows the effect of phosphorothioate substitutions on the catalytic activity of 1049 2'-C-allyl HH ribozyme. A) diagrammatic representation of 1049 hammerhead ribozyme-substrate complex. 1049 U4-C-allyl P=S ribozyme represents a hammerhead containing ribose residues at five positions. The remaining 31 nucleotide positions contain 2'-hydroxyl  
25 group substitutions, wherein 30 nucleotides contain 2'-O-methyl substitutions and one nucleotide (U<sub>4</sub>) contains 2'-C-allyl substitution. Additionally, five nucleotides within the ribozyme, at the 5' and 3' termini, contain

phosphorothioate substitutions. B) shows the ability of ribozyme described in Fig. 17A to decrease the level of stromelysin RNA in rabbit knee.

Figure 18 is a diagrammatic representation of chemically modified ribozymes targeted against stromelysin RNA. 1049 2'-amino P=S Ribozyme represents a hammerhead containing ribose residues at five positions. The remaining 31 nucleotide positions contain 2'-hydroxyl group substitutions, wherein 29 nucleotides contain 2'-O-methyl substitutions and two nucleotides (U<sub>4</sub> and U<sub>7</sub>) contain 2'-amino substitution. Additionally, the 3' end of this ribozyme contains a 3'-3' linked inverted T and four nucleotides at the 5' termini contain phosphorothioate substitutions. Arrow-head indicates the site of RNA cleavage (site 1049). 1363 2'-Amino P=S, Human and Rabbit 1366 2'-Amino P=S ribozymes are identical to the 1049 2'-amino P=S ribozyme except that they are targeted to sites 1363 and 1366 within stromelysin RNAs.

Figure 19 shows 1049 2'-amino P=S ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

Figure 20 shows 1363 2'-amino P=S ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

Figure 21 shows 1366 2'-amino P=S ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

Figures 22a-d are diagrammatic representations of non-limiting examples of base modifications for adenine, guanine, cytosine and uracil, respectively.

Figure 23 is a diagrammatic representation of a position numbered hammerhead ribozyme (according to Hertel *et al.*, *Nucleic Acids Res.* 1992, 20:3252) showing specific substitutions in the catalytic core and substrate binding arms. Compounds 4, 9, 13, 17, 22 and 23 are described in Fig. 24.

Figure 24 is a diagrammatic representation of various nucleotides that can be substituted in the catalytic core of a hammerhead ribozyme.

Figure 25 is a diagrammatic representation of the synthesis of a ribothymidine phosphoramidite.

Figur 26 is a diagrammatic representation of the synthesis of a 5-methylcytidine phosphoramidite.

Figure 27 is a diagrammatic representation of the synthesis of 5-bromouridine phosphoramidite.

5 Figure 28 is a diagrammatic representation of the synthesis of 6-azauridine phosphoramidite.

Figure 29 is a diagrammatic representation of the synthesis of 2,6-diaminopurine phosphoramidite.

10 Figure 30 is a diagrammatic representation of the synthesis of a 6-methyluridine phosphoramidite.

Figure 31 is a representation of a hammerhead ribozyme targeted to site A (HH-A). Site of 6-methyl U substitution is indicated.

15 Figure 32 shows RNA cleavage reaction catalyzed by HH-A ribozyme containing 6-methyl U-substitution (6-methyl-U4). U4, represents a HH-A ribozyme containing no 6-methyl-U substitution.

Figure 33 is a representation of a hammerhead ribozyme targeted to site B (HH-B). Sites of 6-methyl U substitution are indicated.

20 Figure 34 shows RNA cleavage reaction catalyzed by HH-B ribozyme containing 6-methyl U-substitutions at U4 and U7 positions (6-methyl-U4). U4, represents a HH-B ribozyme containing no 6-methyl-U substitution.

Figure 35 is a representation of a hammerhead ribozyme targeted to site C (HH-C). Sites of 6-methyl U substitution are indicated.

25 Figure 36 shows RNA cleavage reaction catalyzed by HH-C ribozyme containing 6-methyl U-substitutions at U4 and U7 positions (6-methyl-U4). U4, represents a HH-C ribozyme containing no 6-methyl-U substitution.

Figure 37 shows 6-methyl-U-substituted HH-A ribozyme-mediated inhibition of rat smooth muscle cell proliferation.

Figure 38 shows 6-methyl-U-substituted HH-C ribozyme-mediated inhibition of stromelysin protein production in human synovial fibroblast cells.

Figure 39 is a diagrammatic representation of the synthesis of pyridin-2-one nucleoside and pyridin-4-one nucleoside phosphoramidite.

5        Figure 40 is a diagrammatic representation of the synthesis of 2-*O*-*t*-Butyldimethylsilyl-5-*O*-dimethoxytrityl-3-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-deoxy-1-phenyl- $\beta$ -D-ribofuranose phosphoramidite.

10       Figure 41 is a diagrammatic representation of the synthesis of pseudouridine, 2,4,6-trimethoxy benzene nucleoside and 3-methyluridine phosphoramidite.

Figure 42 is a diagrammatic representation of the synthesis of dihydrouridine phosphoramidite.

15       Figure 43 A) is diagrammatic representation of a hammerhead ribozyme targeted to site . . . B) shows RNA cleavage reaction catalyzed by hammerhead ribozyme with modified base substitutions at either position 4 or position 7.

20       Figure 44 shows further kinetic characterization of RNA cleavage reactions catalyzed by HH-B ribozyme (A); HH-B with pyridin-4-one substitution at position 7 (B); and HH-B with phenyl substitution at position 7 (C).

Figure 45 is a diagrammatic representation of the synthesis of 2-*O*-*t*-Butyldimethylsilyl-5-*O*-Dimethoxytrityl-3-*O*-(2-Cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-Deoxy-1-Naphthyl- $\beta$ -D-Ribofuranose.

25       Figure 46 is a diagrammatic representation of the synthesis of Synthesis of 2-*O*-*t*-Butyldimethylsilyl-5-*O*-Dimethoxytrityl-3-*O*-(2-Cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-Deoxy-1-(*p*-Aminophenyl)- $\beta$ -D-Ribofuranose.



Figure 47 is a diagrammatic representation of a position numbered hammerhead ribozyme (according to Hertel *et al. Nucleic Acids Res.* 1992, 20, 3252) showing specific substitutions.

5      Figure 48 shows the structures of various 2'-alkyl modified nucleotides which exemplify those of this invention. R groups are alkyl groups, Z is a protecting group.

Figure 49 is a diagrammatic representation of the synthesis of 2'-C-allyl uridine and cytidine.

10      Figure 50 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene uridine.

Figure 51 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene cytidine.

Figure 52 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene adenosine.

15      Figure 53 is a diagrammatic representation of the synthesis of 2'-C-carboxymethylidene uridine, 2'-C-methoxycarboxymethylidene uridine and derivatized amidites thereof. X is CH<sub>3</sub> or alkyl as discussed above, or another substituent.

20      Figure 54 is a diagrammatic representation of the synthesis of 2'-C-allyl uridine and cytidine phosphoramidites.

25      Figure 55 is a diagrammatic representation of the synthesis of 2'-O-alkylthioalkyl nucleosides or non-nucleosides and their phosphoramidites. R is an alkyl as defined above. B is any naturally occurring or modified base bearing any N-protecting group suitable for standard oligonucleotide synthesis (Usman *et al.*, *supra*; Scaringe *et al.*, *supra*), and/or H (non-nucleotide), as described by the publications discussed above. CE is cyanoethyl, DMT is a standard blocking group. Other abbreviations are standard in the art.

Figure 56 is a diagrammatic representation of a hammerhead ribozyme, targeted to site B (HH-B), containing 2'-O-methylthiomethyl substitutions.

Figure 57 shows RNA cleavage activity catalyzed by 2'-O-methylthiomethyl substituted ribozymes. A plot of percent cleaved as a function of time is shown. The reactions were carried out at 37°C in the presence of 40 nM ribozyme, 1 nM substrate and 10 mM MgCl<sub>2</sub>. Control HH-B ribozyme contained the following modifications; 29 positions were modified with 2'-O-methyl, U4 and U7 positions were modified with 2'-amino groups, 5 positions contained 2'-OH groups. These modifications of the control ribozyme have previously been shown not to significantly effect the activity of the ribozyme (Usman et al., 1994 *Nucleic Acids Symposium Series* 31, 163).

Figure 58 is a diagrammatic representation of the synthesis of an abasic deoxyribose or ribose non-nucleotide mimetic phosphoramidite.

Figure 59 is a diagrammatic representation of a hammerhead ribozyme targeted to site B (HH-B). Arrow indicates the cleavage site.

Figure 60 is a diagrammatic representation of HH-B ribozyme containing abasic substitutions (HH-Ba) at various positions. Ribozymes were synthesized as described in the application. "X" shows the positions of abasic substitutions. The abasic substitutions were either made individually or in certain combinations.

Figure 61 shows the *in vitro* RNA cleavage activity of HH-B and HH-Ba ribozymes. All RNA, refers to HHA ribozyme containing no abasic substitution. U4 Abasic, refers to HH-Ba ribozyme with a single abasic (ribose) substitution at position 4. U7 Abasic, refers to HH-Ba ribozyme with a single abasic (ribose) substitution at position 7.

Figure 62 shows *in vitro* RNA cleavage activity of HH-B and HH-Ba ribozymes. Abasic Stem II Loop, refers to HH-Ba ribozyme with four abasic (ribose) substitutions within the loop in stem II.

Figure 63 shows *in vitro* RNA cleavage activity of HH-B and HH-Ba ribozymes. 3'-Inverted Deoxyribose, refers to HH-Ba ribozyme with an inverted deoxyribose (abasic) substitution at its 3' termini.

5 Figure 64 is a diagrammatic representation of a hammerhead ribozyme targeted to site A (HH-A). Target A is involved in the proliferation of mammalian smooth muscle cells. Arrow indicates the site of cleavage. Inactive version of HH-A contains 2 base-substitutions (G5U and A15.1U) that renders the ribozyme catalytically inactive.

10 Figure 65 is a diagrammatic representation of HH-A ribozyme with abasic substitution (HH-Aa) at position 4. X, shows the position of abasic substitution.

Figure 66 shows ribozyme-mediated inhibition of rat aortic smooth muscle cell (RASMC) proliferation. Both HH-A and HH-Aa ribozymes can inhibit the proliferation of RASMC in culture. Catalytically inactive HH-A ribozyme shows inhibition which is significantly lower than active HH-A and  
15 HH-Aa ribozymes.

Figure 67 is a schematic representation of a two pot deprotection protocol with ethylamine (EA).

20 Figure 68 shows a strategy used in synthesizing a hammerhead ribozyme from two halves. X and Y represent reactive moieties that can undergo a chemical reaction to form a covalent bond (represented by the solid curved line).

Figure 69 shows various non-limiting examples of reactive moieties that can be placed in the nascent loop region to form a covalent bond to provide a full-length ribozyme. CH<sub>2</sub> can be any linking chain as described above  
25 including groups such as methylenes, ether, ethylene glycol, thioethers, double bonds, aromatic groups and others; each n independently is an integer from 0 to 10 inclusive and may be the same or different; each R independently is a proton or an alkyl, alkenyl and other functional groups or conjugates such as peptides, steroids, hormones, lipids, nucleic acid sequences and others  
30 that provides nuclease resistance, improved cell association, improved cellular uptake or interacellular localization.

Figure 70 shows non-limiting examples of covalent bonds that can be formed to provide the full length ribozyme. The morpholino group arises from reductive reaction of a dialdehyde, which arises from oxidative cleavage of a ribose at the 3'-end of one half ribozyme with an amine at the 5'-end of the half ribozyme. The amide bond is produced when an acid at the 3'-end of one half ribozyme is coupled to an amine at the 5'-end of the other half ribozyme.

Figure 71 shows non-limiting examples of three ribozymes that were synthesized from coupling reactions of two halves. All three were targeted to the site A of *c-myc* RNA (HH-A). HH-A1 was formed from the reaction of two thiols to provide the disulfide linked ribozyme. HH-A2 and HH-A3 were formed using the morpholino reaction. HH-A2 contains a five atom spacer linking the terminal amine to the 5'-end of the half ribozyme. HH-A3 contains a six carbon spacer linking the terminal amine to the 5'-end of the half ribozyme.

Figure 72 shows comparative cleavage activity of half ribozymes, containing five and six base pair stem II regions, that are not covalently linked vs a full length ribozyme. Assays were carried out under ribozyme excess conditions.

Figure 73 shows comparative cleavage activity of half ribozymes, containing seven and eight base pair stem II regions, that are not covalently linked vs a full length ribozyme. Assays were carried out under ribozyme excess conditions.

Figure 74 shows comparative cleavage assay of HH-A1, HH-A2 and HH-A3 (see Figure 72) formed from crosslinking reactions vs a full length ribozyme control. Assays were carried out under ribozyme excess conditions.

Figure 75. Schematic representation of RNA polymerase III promoter structure. Arrow indicates the transcription start site and the direction of coding region. A, B and C, refer to consensus A, B and C box promoter sequences. I, refers to intermediate cis-acting promoter sequence. PSE, refers to proximal sequence element. DSE, refers to distal sequence element. ATF, refers to activating transcription factor binding element. ?, refers to cis-

acting sequence element that has not been fully characterized: EBER, Epstein-Barr-virus-encoded-RNA. TATA is a box well known in the art.

Figure 76 is a general formula for pol III RNA of this invention.

5 Figure 77 is a diagrammatic representation of a U6-S35 Chimera. The S35 motif and the site of insertion of a desired RNA are indicated. This chimeric RNA transcript is under the control of a U6 small nuclear RNA (snRNA) promoter.

10 Figure 78 is a diagrammatic representation of a U6-S35-ribozyme chimera. The chimera contains a hammerhead ribozyme targeted to site I (HHI).

Figure 79 is a diagrammatic representation of a U6-S35-ribozyme chimera. The chimera contains a hammerhead ribozyme targeted to site II (HHII).

15 Figure 80 shows RNA cleavage reaction catalyzed by a synthetic hammerhead ribozyme (HHI) and by an *in vitro* transcript of U6-S35-HHI hammerhead ribozyme.

Figure 81 shows stability of U6-S35-HHII RNA transcript in 293 mammalian cells as measured by actinomycin D assay.

20 Figure 82 is a diagrammatic representation of an adenovirus VA1 RNA. Various domains within the RNA secondary structure are indicated.

25 Figure 83 A shows a secondary structure model of a VA1-S35 chimeric RNA containing the promoter elements A and B box. The site of insertion of a desired RNA and the S35 motif are indicated. The transcription unit also contains a stable stem (S35-like motif) in the central domain of the VA1 RNA which positions the desired RNA away from the main transcript as an independent domain. 83B shows a VA1-chimera which consists of the terminal 75 nt of a VA1 RNA followed by the HHI ribozyme.

Figure 84 shows a comparison of stability of VA1-chimeric RNA vs VA1-S35-chimeric RNA as measured by actinomycin D assay. VA1-chimera

consists of terminal 75 nt of VA1 RNA followed by HHI ribozyme. VA1-S35-chimera structure and sequence is shown in Figure 83.

### Ribozymes

5 Ribozymes in one aspect of this invention block to some extent stromelysin expression and can be used to treat disease or diagnose such disease. Ribozymes are delivered to cells in culture and to cells or tissues in animal models of osteoarthritis (Hembry et al., 1993 Am. J. Pathol. 143, 628). Ribozyme cleavage of stromelysin encoding mRNAs in these systems may prevent inflammatory cell function and alleviate disease symptoms.

10 Other ribozymes of this invention block to some extent B7-1, B7-2, B7-3 and/or CD40 production and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture, to cells or tissues in animal models of transplantation, autoimmune diseases and/or allergies and to human cells or tissues *ex vivo* or *in vivo*. Ribozyme cleavage of B7-1, B7-2  
15 and/or CD40 encoded mRNAs in these systems may alleviate disease symptoms.

### Target sites

Targets for useful ribozymes can be determined as disclosed in Draper et al supra, Sullivan et al., supra, as well as by Draper et al., WO 95/13380 and  
20 Stinchcomb et al WO 95/23225. Rather than repeat the guidance provided in those documents here, below are provided specific examples of such methods, not limiting to those in the art. Ribozymes to such targets are designed as described in those applications and synthesized to be tested *in vitro* and *in vivo*, as also described. Such ribozymes can also be optimized  
25 and delivered as described therein. While specific examples to mouse, rabbit and other animal RNA are provided, those in the art will recognize that the equivalent human RNA targets described can be used as described below. Thus, the same target may be used, but binding arms suitable for targeting human RNA sequences are present in the ribozyme. Such targets may also  
30 be selected as described below.

The sequence of human and rabbit stromelysin mRNA were screened for accessible sites using a computer folding algorithm. Potential hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables All, AIII, AIV, AVI, AVIII and AIX (All sequences are 5' to 3' in the tables.). While rabbit and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, rabbit targeted ribozymes are useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

10 Similarly, the sequence of human and mouse B7-1, B7-2, B7-3 and/or CD40 mRNAs were screened for optimal ribozyme target sites using a computer folding algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables BII, BIV, BVI, BVIII, BX, BXII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX (All sequences are 5' to 3' in the tables) The nucleotide base position is noted in the Tables as that site to be  
15 cleaved by the designated type of ribozyme. While mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, mouse targeted ribozymes may be useful to test efficacy of action of the ribozyme prior to testing in  
20 humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

Hammerhead or hairpin ribozymes are designed that could bind and are individually analyzed by computer folding (Jaeger et al., 1989 Proc. Natl. Acad. Sci. USA, 86, 7706-7710) to assess whether the ribozyme sequences  
25 fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

30 Referring to Figure 6, mRNA is screened for accessible cleavage sites by the method described generally in Draper WO 93/23569. Briefly, DNA oligonucleotides representing potential hammerhead or hairpin ribozyme cleavage sites are synthesized. A polymerase chain reaction is used to

generate a substrate for T7 RNA polymerase transcription from human or rabbit stromelysin cDNA clones. Labeled RNA transcripts are synthesized *in vitro* from the two templates. The oligonucleotides and the labeled transcripts are annealed, RNaseH is added and the mixtures are incubated for the designated times at 37°C. Reactions are stopped and RNA separated on sequencing polyacrylamide gels. The percentage of the substrate cleaved is determined by autoradiographic quantitation using a PhosphorImaging system. From these data, hammerhead ribozyme sites are chosen as the most accessible.

10 Ribozymes of the hammerhead or hairpin motif are designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above. The ribozymes are chemically synthesized. The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman et al., 1987 J. Am. Chem. Soc., 109, 7845-7854 and in Scaringe et al., 1990 Nucleic Acids Res., 18, 5433-5441; Wincott et al., 1995 Nucleic Acids Res., 23, 2677, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%. Inactive ribozymes were synthesized by substituting a U for G<sub>5</sub> and a U for A<sub>14</sub> (numbering from Hertel et al., 1992 Nucleic Acids Res., 20, 3252). Hairpin ribozymes are synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 Nucleic Acids Res., 20, 2835-2840). All ribozymes are modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 25 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992 TIBS 17, 34 and Beigelman et al., 1995 J. Biol. Chem. 270, 25702). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; See Stinchcomb et al, supra) and are resuspended in water.

30 The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables AV, AVII, AVIII and AIX and in Tables BIII, BV, BVI, BVII, BIX, BXI, BXIII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the



binding arms) is altered to affect activity. For example, stem loop II sequence of hammerhead ribozymes listed in Tables AV and AVII (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. Similarly, stem-loop AIV sequence of hairpin ribozymes listed in Tables AVI and AVII (5'-CACGUUGUG-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. The sequences listed in Tables AV, AVII, AVIII and AIX may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

#### Optimizing Ribozyme Activity

Ribozyme activity can be optimized as described by Stinchcomb *et al.*, supra. The details will not be repeated here, but include altering the length of the ribozyme binding arms (stems I and III, see Figure 2c), or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see *e.g.*, Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 Nature 344, 565; Pieken *et al.*, 1991 Science 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162, as well as Stinchcomb *et al.*, supra, Sproat, European Patent Application 92110298.4 and U.S. Patent 5,334,711; Jennings *et al.*, WO 94/13688 and Beigelman *et al.*, supra which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules). Modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements.

Sullivan, *et al.*, supra, describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. For some

indications, ribozymes may be directly delivered *ex vivo* to cells or tissues with or without the aforementioned vehicles. Alternatively, the RNA/vehicle combination is locally delivered by direct inhalation, by direct injection or by use of a catheter, infusion pump or stent. Other routes of delivery include, but are not limited to, intravascular, intramuscular, subcutaneous or joint injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Sullivan et al., supra and Draper et al., supra which have been incorporated by reference herein.

10 In another preferred embodiment, the ribozyme is administered to the site of B7-1, B7-2, B7-3 and/or CD40 expression (APC) in an appropriate liposomal vesicle. APCs isolated from donor (for example) are treated with the ribozyme preparation (or other nucleic acid therapeutics) *ex vivo* and the treated cells are infused into recipient. Alternatively, cells, tissues or organs are directly treated with nucleic acids of the present invention prior to transplantation into a recipient.

Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990 Proc. Natl. Acad. Sci. U S A, 87, 6743-7; Gao and Huang 1993 Nucleic Acids Res., 21, 2867-72; Lieber et al., 1993 Methods Enzymol., 217, 47-66; Zhou et al., 1990 Mol. Cell. Biol., 10, 4529-37). Several investigators have demonstrated that ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet et al., 1992 Antisense Res. Dev., 2, 3-15; Ojwang et al., 1992 Proc. Natl. Acad. Sci. U S A, 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Yu et al., 1993 Proc. Natl. Acad. Sci. U S A, 90, 6340-4; L'Huillier et al., 1992 EMBO J. 11, 4411-8; Lisiewicz et

al., 1993 Proc. Natl. Acad. Sci. U. S. A., 90, 8000-4; Thompson *et al.*, *supra*).  
The above ribozyme transcription units can be incorporated into a variety of  
vectors for introduction into mammalian cells, including but not restricted to,  
plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-  
5 associated vectors), or viral RNA vectors (such as retroviral or alphavirus  
vectors).

In a preferred embodiment of the invention, a transcription unit  
expressing a ribozyme that cleaves stromelysin RNA is inserted into a plasmid  
DNA vector or an adenovirus DNA virus or adeno-associated virus (AAV)  
10 vector. Both viral vectors have been used to transfer genes to the lung and  
both vectors lead to transient gene expression (Zabner *et al.*, 1993 Cell 75,  
207; Carter, 1992 Curr. Opin. Biotech. 3, 533). The adenovirus vector is  
delivered as recombinant adenoviral particles. The DNA may be delivered  
alone or complexed with vehicles (as described for RNA above). The  
15 recombinant adenovirus or AAV particles are locally administered to the site of  
treatment, *e.g.*, through incubation or inhalation *in vivo* or by direct application  
to cells or tissues *ex vivo*.

Specifically useful modifications, optimization and synthetic methods will  
now be described.

#### 20 Base Modifications

The following discussion of relevant art is dependent on the diagram  
shown in Figure 1, in which the numbering of various nucleotides in a  
hammerhead ribozyme is provided.

Odai *et al.*, *FEBS* 1990, 267:150, state that substitution of guanosine (G)  
25 at position 5 of a hammerhead ribozyme for inosine greatly reduces catalytic  
activity, suggesting "the importance of the 2-amino group of this guanosine for  
catalytic activity."

Fu and McLaughlin, *Proc. Natl. Acad. Sci. (USA)* 1992, 89:3985, state  
that deletion of the 2-amino group of the guanosine at position 5 of a  
30 hammerhead ribozyme, or deletion of either of the 2'-hydroxyl groups at

position 5 or 8, resulted in ribozymes having a decrease in cleavage efficiency.

5 Fu and McLaughlin, *Biochemistry* 1992, 31:10941, state that substitution of 7-deazaadenosine for adenosine residues in a hammerhead ribozyme can cause reduction in cleavage efficiency. They state that the "results suggest that the N<sup>7</sup>-nitrogen of the adenosine (A) at position 6 in the hammerhead ribozyme/substrate complex is critical for efficient cleavage activity." They go on to indicate that there are five critical functional groups located within the tetrameric sequence GAUG in the hammerhead ribozyme.

10 Slim and Gait, 1992, *BBRC* 183, 605, state that the substitution of guanosine at position 12, in the core of a hammerhead ribozyme, with inosine inactivates the ribozyme.

15 Tuschl *et al.*, 1993 *Biochemistry* 32, 11658, state that substitution of guanosine residues at positions 5, 8 and 12, in the catalytic core of a hammerhead, with inosine, 2-aminopurine, xanthosine, isoguanosine or deoxyguanosine cause significant reduction in the catalytic efficiency of a hammerhead ribozyme.

20 Fu *et al.*, 1993 *Biochemistry* 32, 10629, state that deletion of guanine N<sup>7</sup>, guanine N<sup>2</sup> or the adenine N<sup>6</sup>-nitrogen within the core of a hammerhead ribozyme causes significant reduction in the catalytic efficiency of a hammerhead ribozyme.

25 Grasby *et al.*, 1993 *Nucleic Acids Res.* 21, 4444, state that substitution of guanosine at positions 5, 8 and 12 positions within the core of a hammerhead ribozyme with O<sup>6</sup>-methylguanosine results in an approximately 75-fold reduction in k<sub>cat</sub>.

Seela *et al.*, 1993 *Helvetica Chimica Acta* 76, 1809, state that substitution of adenine at positions 13, 14 and 15, within the core of a hammerhead ribozyme, with 7-deazaadenosine does not significantly decrease the catalytic efficiency of a hammerhead ribozyme.

Adams *et al.*, 1994 *Tetrahedron Letters* 35, 765, state that substitution of uracil at position 17 within the hammerhead ribozyme-substrate complex with 4-thiouridine results in a reduction in the catalytic efficiency of the ribozyme by 50 percent.

5        Ng *et al.*, 1994 *Biochemistry* 33, 12119, state that substitution of adenine at positions 6, 9 and 13 within the catalytic core of a hammerhead ribozyme with isoguanosine, significantly decreases the catalytic activity of the ribozyme.

10        Jennings *et al.*, U.S. Patent 5,298,612, indicate that nucleotides within a "minizyme" can be modified. They state-

15                "Nucleotides comprise a base, sugar and a monophosphate group. Accordingly, nucleotide derivatives or modifications may be made at the level of the base, sugar or monophosphate groupings..... Bases may be substituted with various groups, such as halogen, hydroxy, amine, alkyl, azido, nitro, phenyl and the like."

20        WO93/23569, WO95/06731, WO95/04818, and WO95/133178 describe various modifications that can be introduced into ribozyme structures.

25        This invention relates to production of enzymatic RNA molecules or ribozymes having enhanced or reduced binding affinity and enhanced enzymatic activity for their target nucleic acid substrate by inclusion of one or more modified nucleotides in the substrate binding portion of a ribozyme such as a hammerhead, hairpin, VS ribozyme or hepatitis delta virus derived  
30        ribozyme. Applicant has recognized that only small changes in the extent of base-pairing or hydrogen bonding between the ribozyme and substrate can have significant effect on the enzymatic activity of the ribozyme on that substrate. Thus, applicant has recognized that a subtle alteration in the extent  
35        of hydrogen bonding along a substrate binding arm of a ribozyme can be used to improve the ribozyme activity compared to an unaltered ribozyme containing no such altered nucleotide. Thus, for example, a guanosine base may be replaced with an inosine to produce a weaker interaction between a ribozyme and its substrate, or a uracil may be replaced with a bromouracil (BrU) to increase the hydrogen bonding interaction with an adenosine. Other

examples of alterations of the four standard ribonucleotide bases are shown in Figures 22a-d with weaker or stronger hydrogen bonding abilities shown in each figure.

In addition, applicant has determined that base modification within some catalytic core nucleotides maintains or enhances enzymatic activity compared to an unmodified molecule. Such nucleotides are noted in Figure 23. Specifically, referring to Figure 23, the preferred sequence of a hammerhead ribozyme in a 5' to 3' direction of the catalytic core is CUG ANG A G•C GAA A, wherein N can be any base or may lack a base (abasic); G•C is a base-pair. The nature of the base-paired stem II (Figures 1, 2 and 23) and the recognition arms of stems I and III are variable. In this invention, the use of base-modified nucleotides in those regions that maintain or enhance the catalytic activity and/or the nuclease resistance of the hammerhead ribozyme are described. (Bases which can be modified include those shown in capital letters).

Examples of base-substitutions useful in this invention are shown in Figure 22, 24-30, 39-43, 45-46. In preferred embodiments cytidine residues are substituted with 5-alkylcytidines (e.g., 5-methylcytidine, Figure 24, R=CH<sub>3</sub>, 9), and uridine residues with 5-alkyluridines (e.g., ribothymidine (Figure 24, R=CH<sub>3</sub>, 4) or 5-halouridine (e.g., 5-bromouridine, Figure 24, X=Br, 13) or 6-azapyrimidines (Figure 24, 17) or 6-alkyluridine (Figure 30). Guanosine or adenosine residues may be replaced by diaminopurine residues (Figure 24, 22) in either the core or stems. In those bases where none of the functional groups are important in the complexing of magnesium or other functions of a ribozyme, they are optionally replaced with a purine ribonucleoside (Figure 24, 23), which significantly reduces the complexity of chemical synthesis of ribozymes, as no base-protecting group is required during chemical incorporation of the purine nucleus. Furthermore, as discussed above, base-modified nucleotides may be used to enhance the specificity or strength of binding of the recognition arms with similar modifications. Base-modified nucleotides, in general, may also be used to enhance the nuclease resistance of the catalytic nucleic acids in which they are incorporated. These modifications within the hammerhead ribozyme motif are meant to be non-limiting example. Those skilled in the art will recognize that other ribozyme

motifs with similar modifications can be readily synthesized and are within the scope of this invention.

Substitutions of sugar moieties as described in the art cited above, may also be made to enhance catalytic activity and/or nuclease stability.

- 5       The invention provides ribozymes having increased enzymatic activity in vitro and in vivo as can be measured by standard kinetic assays. Thus, the kinetic features of the ribozyme are enhanced by selection of appropriate modified bases in the substrate binding arms. Applicant recognizes that while strong binding to a substrate by a ribozyme enhances specificity, it may also
- 10       prevent separation of the ribozyme from the cleaved substrate. Thus, applicant provides means by which optimization of the base pairing can be achieved. Specifically, the invention features ribozymes with modified bases with enzymatic activity at least 1.5 fold (preferably 2 or 3 fold) or greater than the unmodified corresponding ribozyme. The invention also features a
- 15       method for optimizing the kinetic activity of a ribozyme by introduction of modified bases into a ribozyme and screening for those with higher enzymatic activity. Such selection may be in vitro or in vivo. By enhanced activity is meant to include activity measured in vivo where the activity is a reflection of both catalytic activity and ribozyme stability. In this invention, the product of
- 20       these properties is increased or not significantly (less than 10 fold) decreased in vivo compared to an all RNA ribozyme.

By "enzymatic portion" is meant that part of the ribozyme essential for cleavage of an RNA substrate.

- 25       By "substrate binding arm" is meant that portion of a ribozyme which is complementary to (*i.e.*, able to base-pair with) a portion of its substrate. Generally, such complementarity is 100%, but can be less if desired. For example, as few as 10 bases out of 14 may be base-paired. Such arms are shown generally in Figures 1-3 as discussed below. That is, these arms contain sequences within a ribozyme which are intended to bring ribozyme
- 30       and target RNA together through complementary base-pairing interactions; *e.g.*, ribozyme sequences within stems I and III of a standard hammerhead ribozyme make up the substrate-binding domain (see Figure 1).

By "unmodified nucleotide base" is meant one of the bases adenine, cytosine, guanosine, uracil joined to the 1' carbon of  $\beta$ -D-ribo-furanose. The sugar also has a phosphate bound to the 5' carbon. These nucleotides are bound by a phosphodiester between the 3' carbon of one nucleotide and th  
5 5' carbon of the next nucleotide to form RNA.

By "modified nucleotide base" is meant any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base which has an effect on the ability of that base to hydrogen bond with its normal complementary base, either by increasing the strength of the hydrogen  
10 bonding or by decreasing it (*e.g.*, as exemplified above for inosine and bromouracil). Other examples of modified bases include those shown in Figures 22a-d and other modifications well known in the art, including heterocyclic derivatives and the like.

In preferred embodiments the modified ribozyme is a hammerhead,  
15 hairpin VS ribozyme or hepatitis delta virus derived ribozyme, and the hammerhead ribozyme includes between 32 and 40 nucleotide bases. The selection of modified bases is most preferably chosen to enhance the enzymatic activity (as observed in standard kinetic assays designed to measure the kinetics of cleavage) of the selected ribozyme, *i.e.*, to enhance  
20 the rate or extent of cleavage of a substrate by the ribozyme, compared to a ribozyme having an identical nucleotide base sequence without any modified base.

By "kinetic assays" or "kinetics of cleavage" is meant an experiment in which the rate of cleavage of target RNA is determined. Often a series of  
25 assays are performed in which the concentrations of either ribozyme or substrate are varied from one assay to the next in order to determine the influence of that parameter on the rate of cleavage.

By "rate of cleavage" is meant a measure of the amount of target RNA cleaved as a function of time.

30 Enzymatic nucleic acid having a hammerhead configuration and modified bases which maintain or enhance enzymatic activity are provided. Such nucleic acid is also generally more resistant to nucleases than



unmodified nucleic acid. By "modified bases" in this aspect is meant those shown in Figure 22 A-D, and 24, 30, and 42B or their equivalents; such bases may be used within the catalytic core of the enzyme as well as in the substrate-binding regions. In particular, the invention features modified ribozymes having a base substitution selected from pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyluracil, dihydrouracil, naphthyl, 6-methyl-uracil and aminophenyl. As noted above, substitution in the core may decrease *in vitro* activity but enhances stability. Thus, *in vivo* the activity may not be significantly lowered. As exemplified herein such ribozymes are useful *in vivo* even if active over all is reduced 10 fold. Such ribozymes herein are said to "maintain" the enzymatic activity on all RNA ribozyme.

Small scale synthesis were conducted on a 394 Applied Biosystems, Inc. synthesizer using a modified 2.5  $\mu$ mol scale protocol with a 5 min coupling step for alkylsilyl protected nucleotides and 2.5 min coupling step for 2'-O-methylated nucleotides. Table CII outlines the amounts, and the contact times, of the reagents used in the synthesis cycle. A 6.5-fold excess (163  $\mu$ L of 0.1 M = 16.3  $\mu$ mol) of phosphoramidite and a 24-fold excess of S-ethyl tetrazole (238  $\mu$ L of 0.25 M = 59.5  $\mu$ mol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, were 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer: detritylation solution was 2% TCA in methylene chloride (ABI); capping was performed with 16% N-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution was 16.9 mM I<sub>2</sub>, 49 mM pyridine, 9% water in THF (Millipore). B & J Synthesis Grade acetonitrile was used directly from the reagent bottle. S-Ethyl tetrazole solution (0.25 M in acetonitrile) was made up from the solid obtained from American International Chemical, Inc.

Deprotection of the RNA was performed as follows. The polymer-bound oligoribonucleotide, trityl-off, was transferred from the synthesis column to a 4mL glass screw top vial and suspended in a solution of methylamine (MA) at 65 °C for 10 min. After cooling to -20 °C, the supernatant was removed from the polymer support. The support was washed three times with 1.0 mL of

EtOH:MeCN:H<sub>2</sub>O/3:1:1, vortexed and the supernatant was then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, were dried to a white powder.

5 The base-deprotected oligoribonucleotide was resuspended in anhydrous TEA•HF/NMP solution (250 µL of a solution of 1.5mL *N*-methylpyrrolidinone, 750 µL TEA and 1.0 mL TEA•3HF to provide a 1.4M HF concentration) and heated to 65°C for 1.5 h. The resulting, fully deprotected, oligomer was quenched with 50 mM TEAB (9 mL) prior to anion exchange desalting.

10 For anion exchange desalting of the deprotected oligomer, the TEAB solution was loaded onto a Qiagen 500<sup>®</sup> anion exchange cartridge (Qiagen Inc.) that was prewashed with 50 mM TEAB (10 mL). After washing the loaded cartridge with 50 mM TEAB (10 mL), the RNA was eluted with 2 M TEAB (10 mL) and dried down to a white powder.

15 Inactive hammerhead ribozymes were synthesized by substituting a U for G<sub>5</sub> and a U for A<sub>14</sub> (numbering from (Hertel, K. J., *et al.*, 1992, *Nucleic Acids Res.*, 20, 3252)).

The average stepwise coupling yields were >98% (Wincott *et al.*, 1995 *Nucleic Acids Res.* 23, 2677-2684).

20 Hairpin ribozymes are synthesized either as one part or in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 *Nucleic Acids Res.*, 20, 2835-2840).

Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; See Stinchcomb *et al.*, International PCT Publication No. WO 95/23225, and are resuspended in water.

25 Various modifications to ribozyme structure can be made to enhance the utility of ribozymes. Such modifications will enhance shelf-life, half-life *in vitro*, stability, and ease of introduction of such ribozymes to the target site, *e.g.*, to enhance penetration of cellular membranes, and confer the ability to recognize and bind to targeted cells.

Examples of such ribozymes are provided in Usman et al., WO 95/13378 and below.

2'-deoxy-2'-nucleotides

Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 Nature 344, 565; Pieken *et al.*, 1991 Science 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162, as well as Stinchcomb *et al.*, *supra*, Sproat, European Patent Application 92110298.4 and U.S. Patent 5,334,711; Jennings *et al.*, WO 94/13688 and Beigelman *et al.*, *supra* which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules. Usman *et al.* also describe various required ribonucleotides in a ribozyme, and methods by which such nucleotides can be defined. De Mesmaeker *et al.* Syn. Lett. 1993, 677-680 (not admitted to be prior art to the present invention) describes the synthesis of certain 2'-C-alkyl uridine and thymidine derivatives. They conclude that "...their use in an antisense approach seems to be very limited."

This invention relates to the use of 2'-deoxy-2'-alkylnucleotides in oligonucleotides, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and also as antisense oligonucleotides. As the term is used in this application, 2'-deoxy-2'-alkylnucleotide-containing enzymatic nucleic acids are catalytic nucleic acid molecules that contain 2'-deoxy-2'-alkylnucleotide components replacing, but not limited to, double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

Also within the invention are 2'-deoxy-2'-alkylnucleotides which may be present in enzymatic nucleic acid or even in antisense oligonucleotides. Contrary to the findings of De Mesmaeker *et al.* applicant has found that such

nucl otides are useful since they enhance the stability of the antisense or enzymatic molecule, and can be used in locations which do not affect the desired activity of the molecule. That is, while the presence of the 2'-alkyl group may reduce binding affinity of the oligonucleotide containing this modification, if that moiety is not in an essential base pair forming region then the enhanced stability that it provides to the molecule is advantageous. In addition, while the reduced binding may reduce enzymatic activity, the enhanced stability may make the loss of activity of less consequence. Thus, for example, if a 2'-deoxy-2'-alkyl-containing molecule has 10% the activity of the unmodified molecule, but has 10-fold higher stability *in vivo* then it has utility in the present invention. The same analysis is true for antisense oligonucleotides containing such modifications. The invention also relates to novel intermediates useful in the synthesis of such nucleotides and oligonucleotides (examples of which are shown in the Figures 48-54), and to methods for their synthesis.

Thus, the invention features 2'-deoxy-2'-alkyl nucleotides, that is a nucleotide base having at the 2'-position on the sugar molecule an alkyl moiety, and in preferred embodiments features those where the nucleotide is not uridine or thymidine. That is, the invention preferably includes all those nucleotides useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed above.

Examples of various alkyl groups useful in this invention are shown in Figure 48, where each R group is any alkyl. These examples are not limiting in the invention. Specifically, an "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO<sub>2</sub> or N(CH<sub>3</sub>)<sub>2</sub>, amino, or SH. The term also includes alkenyl groups which are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may

be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO<sub>2</sub>, halogen, N(CH<sub>3</sub>)<sub>2</sub>, amino, or SH. The term "alkyl" also includes alkynyl groups which have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO<sub>2</sub> or N(CH<sub>3</sub>)<sub>2</sub>, amino or SH.

10 The term "alkyl" does not include alkoxy groups which have an "-O-alkyl" group, where "alkyl" is defined as described above, where the O is adjacent the 2'-position of the sugar molecule.

Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group which has at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 2'-deoxy-2'-alkylnucleotides (preferably not a 2'-alkyl- uridine or thymidine); e.g. enzymatic nucleic acids having a 2'-deoxy-2'-alkylnucleotide; and a method for producing an

enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic molecule with at least one nucleotide having at its 2'-position an alkyl group. In other related aspects, the invention features 2'-deoxy-2'-alkylnucleotide triphosphates.

5 These triphosphates can be used in standard protocols to form useful oligonucleotides of this invention.

The 2'-alkyl derivatives of this invention provide enhanced stability to the oligonucleotides containing them. While they may also reduce absolute activity in an *in vitro* assay they will provide enhanced overall activity *in vivo*.

10 Below are provided assays to determine which such molecules are useful. Those in the art will recognize that equivalent assays can be readily devised.

In another aspect, the invention features hammerhead motifs having enzymatic activity having ribonucleotides at locations shown in Figure 47 at 5, 6, 8, 12, and 15.1, and having substituted ribonucleotides at other positions in

15 the core and in the substrate binding arms if desired. (The term "core" refers to positions between bases 3 and 14 in Figure 47, and the binding arms correspond to the bases from the 3'-end to base 15.1, and from the 5'-end to base 2). Applicant has found that use of ribonucleotides at these five locations in the core provide a molecule having sufficient enzymatic activity even when

20 modified nucleotides are present at other sites in the motif. Other such combinations of useful ribonucleotides can be determined as described by Usman *et al. supra*.

#### 2'-O-alkylthioalkyl and 2'-C-alkylthioalkyl containing nucleic acids

Medina *et al.*, 1988 *Tetrahedron Letters* 29, 3773, describe a method to

25 convert alcohols to methylthiomethyl ethers.

Matteucci *et al.*, 1990 *Tetrahedron Letters*, 31, 2385, report the synthesis of 3'-5'-methylene bond via a methylthiomethyl precursor.

Veeneman *et al.*, 1990 *Recl. Trav. Chim. Pays-Bas* 109, 449, report the synthesis of 3'-O-methylthiomethyl deoxynucleoside during the synthesis of a

30 dimer containing 3'-5'-methylene bond.

Jones et al., 1993 *J. Org. Chem.* 58, 2983, report the use of 3'-O-methylthiomethyl deoxynucleoside to synthesize a dimer containing a 3'-thioformacetal internucleoside linkages. The paper also describes a method to synthesize phosphoramidites for DNA synthesis.

- 5       Zavgorodny et al., 1991 *Tetrahedron Letters* 32, 7593, describe a method to synthesize a nucleoside containing methylthiomethyl modification.

10       This invention relates to the incorporation of 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotides or non-nucleotides into nucleic acids, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and also as antisense oligonucleotides.

15       As the term is used in this application, 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotide or non-nucleotide-containing enzymatic nucleic acids are catalytic nucleic molecules that contain 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotide or non-nucleotides components replacing one or  
20       more bases or regions including, but not limited to, those bases in double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can  
25       also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

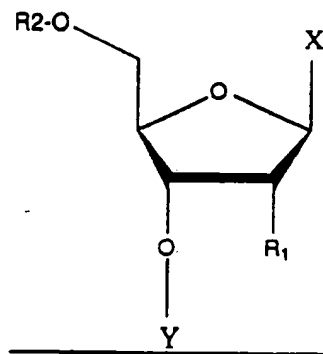
25       Also within the invention are 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotides or non-nucleotides which may be present in enzymatic nucleic acid or in antisense oligonucleotides or 2-5A antisense chimera. Such  
30       nucleotides or non-nucleotides are useful since they enhance the activity of the antisense or enzymatic molecule. The invention also relates to novel intermediates useful in the synthesis of such nucleotides or non-nucleotides and oligonucleotides (examples of which are shown in the Figures), and to methods for their synthesis.

30       Thus, the invention features 2'-O-alkylthioalkyl nucleosides or non-nucleosides, that is a nucleoside or non-nucleosides having at the 2'-position on the sugar molecule a 2'-O-alkylthioalkyl moiety. In a related aspect, the

invention also features 2'-O-alkylthioalkyl nucleotides or non-nucleotides. That is, the invention preferably includes those nucleotides or non-nucleotides having 2' substitutions as noted above useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed above.

The term non-nucleotide refers to any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is abasic in that it does not contain a commonly recognized nucleotide base, such as adenine, guanine, cytosine, uracil or thymine. It may have substitutions for a 2' or 3' H or OH as described in the art. See Eckstein et al. and Usman et al., *supra*.

The term nucleotide refers to the regular nucleotides (A, U, G, T and C) and modified nucleotides such as 6-methyl U, inosine, 5-methyl C and others. Specifically, the term "nucleotide" is used as recognized in the art to include natural bases, and modified bases well known in the art. Such bases are generally located at the 1' position of a sugar moiety. The term "non-nucleotide" as used herein to encompass sugar moieties lacking a base or having other chemical groups in place of a base at the 1' position. Such molecules generally include those having the general formula:



wherein, R<sub>1</sub> represents 2'-O-alkylthioalkyl or 2'-C-alkylthioalkyl; X represents a base or H; Y represents a phosphorus-containing group; and R<sub>2</sub> represents H, DMT or a phosphorus-containing group (Figure 55).



Phosphorus-containing group is generally a phosphate, thiophosphate, H-phosphonate, methylphosphonate, phosphoramidite or other modified group known in the art.

5 In another aspect, the invention features 2'-C-alkylthioalkyl nucleosides or non-nucleosides, that is a nucleotide or a non-nucleotide residue having at the 2'-position on the sugar molecule a 2'-C-alkylthioalkyl moiety. In a related aspect, the invention also features 2'-C-alkylthioalkyl nucleotides or non-nucleotides. That is, the invention preferably includes all those 2' modified nucleotides or non-nucleotides useful for making enzymatic nucleic acids or  
10 antisense molecules as described above that are not described by the art discussed above.

Specifically, an "alkyl" group is as defined above, except that the term includes 2'-O-alkyl moieties.

15 In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotides or non-nucleotides; e.g. enzymatic nucleic acids having a 2'-O-methylthiomethyl and/or 2'-C-alkylthioalkyl nucleotides or non-nucleotides; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule,  
20 by forming the enzymatic molecule with at least one nucleotide or a non-nucleotide moiety having at its 2'-position an 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl group.

In other related aspects, the invention features 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotide triphosphates. These triphosphates can be  
25 used in standard protocols to form useful oligonucleotides of this invention.

The 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl derivatives of this invention provide enhanced activity and stability to the oligonucleotides containing them.

30 In yet another preferred embodiment, the invention features oligonucleotides having one or more 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl abasic (non-nucleotide) moieties. For example, enzymatic

nucleic acids having a 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl abasic moiety; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic molecule with at least one position having at its 2'-  
5 position an 2'-O-alkylthioalkyl or 2'-C-alkylthioalkyl group.

In related embodiments, the invention features enzymatic nucleic acids containing one or more 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl substitutions either in the enzymatic portion, substrate binding portion or both, as long as the catalytic activity of the ribozyme is not significantly decreased.

10 In yet another preferred embodiment, the invention features the use of 2'-O-alkylthioalkyl moieties as protecting groups for 2'-hydroxyl positions of ribofuranose during nucleic acid synthesis.

While this invention is applicable to all oligonucleotides, applicant has found that the modified molecules of this invention are particularly useful for  
15 enzymatic RNA molecules. Thus, below is provided examples of such molecules. Those in the art will recognize that equivalent procedures can be used to make other molecules without such enzymatic activity. Specifically, Figure 1 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided.

20 Referring to Figure 1, the preferred sequence of a hammerhead ribozyme in a 5'- to 3'-direction of the catalytic core is CUGANGAG [base paired with] CGAAA. In this invention, the use of 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl substituted nucleotides or non-nucleotides that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead  
25 ribozyme is described. Substitutions of any nucleotide with any of the modified nucleotides or non-nucleotides discussed above are possible. Usman *et al.*, *supra* and Sproat *et al.*, *supra* as well as other publications indicate those bases that can be substituted in noted ribozyme motifs. Those in the art can thus determine those bases that may be substituted as described  
30 herein with beneficial retainment of enzymatic activity and stability.

Non-nucleotides

Usman, et al., WO 93/15187 in discussing modified structures in ribozymes states:

5           It should be understood that the linkages between  
the building units of the polymeric chain may be  
linkages capable of bridging the units together for  
either in vitro or in vivo. For example the linkage  
may be a phosphorous containing linkage, e.g.,  
10       phosphodiester or phosphothioate, or may be a  
nitrogen containing linkage, e.g., amide. It should  
further be understood that the chimeric polymer  
may contain non-nucleotide spacer molecules  
along with its other nucleotide or analogue units.  
15       Examples of spacer molecules which may be used  
are described in Nielsen et al. Science, 254:1497-  
1500 (1991).

Jennings et al., WO 84/13688 while discussing hammerhead ribozymes lacking the usual stem II base-paired region state:

20           One or more ribonucleotides and/or  
deoxyribonucleotides of the group (X)<sub>m</sub>. [stem II]  
may be replaced, for example, with a linker  
selected from optionally substituted  
polyphosphodiester (such as poly(1-phospho-3-  
25       propanol)), optionally substituted alkyl, optionally  
substituted polyamide, optionally substituted glycol,  
and the like. Optional substituents are well known  
in the art, and include alkoxy (such as methoxy,  
ethoxy and propoxy), straight or branch chain lower  
alkyl such as C<sub>1</sub> - C<sub>5</sub> alkyl), amine, aminoalkyl  
30       (such as amino C<sub>1</sub> - C<sub>5</sub> alkyl), halogen (such as F,  
Cl and Br) and the like. The nature of optional  
substituents is not of importance, as long as the  
resultant endonuclease is capable of substrate  
cleavage.

35           Additionally, suitable linkers may comprise  
polycyclic molecules, such as those containing  
phenyl or cyclohexyl rings. The linker (L) may be a  
polyether such as polyphosphopropanediol,  
polyethyleneglycol, a bifunctional polycyclic  
40       molecule such as a bifunctional pentalene, indene,  
naphthalene, azulene, heptalene, biphenylene,  
asymindacene, sym-indacene, acenaphthylene,  
fluorene, phenalene, phenanthrene, anthracene,  
fluoranthene, acephenathrylene, aceanthrylene,

5 triphenylene, pyrene, chrysene, naphthacene, thianthrene, isobenzofuran, chromene, xanthene, phenoxathiin, indolizine, isoindole, 3-H-indole, indole, 1-H-indazole, 4-H-quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, 4- $\alpha$ H-carbazole, carbazole, B-carboline, phenanthridine, acridine, perimidine, phenanthroline, phenazine, phenothiazine, phenoxazine, which polycyclic  
10 compound may be substituted or modified, or a combination of the polyethers and the polycyclic molecules.

The polycyclic molecule may be substituted of polysubstituted with C<sub>1</sub>-C<sub>5</sub> alkyl, alkenyl, hydroxyalkyl, halogen or haloalkyl group or with O-A or CH<sub>2</sub>-O-A wherein A is H or has the formula CONR'R" wherein R' and R" are the same or different and are hydrogen or a substituted or unsubstituted C<sub>1</sub>-C<sub>6</sub> alkyl, aryl, cycloalkyl, or heterocyclic group; or A has the formula -M-NR'R" wherein R' and R" are the same or different and are hydrogen, or a C<sub>1</sub>-C<sub>5</sub> alkyl, alkenyl, hydroxyalkyl, or haloalkyl group wherein the halo atom is fluorine, chlorine, bromine, or iodine atom; and -M- is an organic moiety having 1 to 10 carbon atoms and is a branched or straight chain alkyl, aryl, or cycloalkyl group.  
15 20 25

In one embodiment, the linker is tetraphosphoropropanediol or pentaphosphoropropanediol. In the case of polycyclic molecules there will be preferably 18 or more atoms bridging the nucleic acids. More preferably their will be from 30 to 50 atoms bridging, see for Example 5. In another embodiment the linker is a bifunctional carbazole or bifunctional carbazole linked to one or more polyphosphoropropanediol.  
30 35

Such compounds may also comprise suitable functional groups to allow coupling through reactive groups on nucleotides."  
40

This invention concerns the use of non-nucleotide molecules as spacer elements at the base of double-stranded nucleic acid (e.g., RNA or DNA) stems (duplex stems) or more preferably, in the single-stranded regions, catalytic core, loops, or recognition arms of enzymatic nucleic acids. Duplex  
45

stems are ubiquitous structural elements in enzymatic RNA molecules. To facilitate the synthesis of such stems, which are usually connected via single-stranded nucleotide chains, a base or base-pair mimetic may be used to reduce the nucleotide requirement in the synthesis of such molecules, and to confer nuclease resistance (since they are non-nucleic acid components). This also applies to both the catalytic core and recognition arms of a ribozyme. In particular abasic nucleotides (i.e., moieties lacking a nucleotide base, but having the sugar and phosphate portions) can be used to provide stability within a core of a ribozyme, e.g., at U4 or N7 of a hammerhead structure shown in Figure 1.

Thus, the invention features an enzymatic nucleic acid molecule having one or more non-nucleotide moieties, and having enzymatic activity to cleave an RNA or DNA molecule.

Examples of such non-nucleotide mimetics are shown in Figure 58 and their incorporation into hammerhead ribozymes is shown in Figure 60. These non-nucleotide linkers may be either polyether, polyamine, polyamide, or polyhydrocarbon compounds. Specific examples include those described by Seela and Kaiser, *Nucleic Acids Res.* 1990, 18:6353 and *Nucleic Acids Res.* 1987, 15:3113; Cload and Schepartz, *J. Am. Chem. Soc.* 1991, 113:6324; Richardson and Schepartz, *J. Am. Chem. Soc.* 1991, 113:5109; Ma et al., *Nucleic Acids Res.* 1993, 21:2585 and *Biochemistry* 1993, 32:1751; Durand et al., *Nucleic Acids Res.* 1990, 18:6353; McCurdy et al., *Nucleosides & Nucleotides* 1991, 10:287; Jäschke et al., *Tetrahedron Lett.* 1993, 34:301; Ono et al., *Biochemistry* 1991, 30:9914; Arnold et al., International Publication No. WO 89/02439 entitled "Non-nucleotide Linking Reagents for Nucleotide Probes"; and Ferentz and Verdine, *J. Am. Chem. Soc.* 1991, 113:4000, all hereby incorporated by reference herein.

In preferred embodiments, the enzymatic nucleic acid includes one or more stretches of RNA, which provide the enzymatic activity of the molecule, linked to the non-nucleotide moiety.

In preferred embodiments, the enzymatic nucleic acid includes one or more stretches of RNA, which provide the enzymatic activity of the molecule,

linked to the non-nucleotide moiety. The necessary ribonucleotide components are known in the art, see, e.g., Usman, supra and Usman et al., Nucl. Acid. Symp. Genes 31:163, 1994.

5 As the term is used in this application, non-nucleotide-containing enzymatic nucleic acid means a nucleic acid molecule that contains at least one non-nucleotide component which replaces a portion of a ribozyme, e.g., but not limited to, a double-stranded stem, a single-stranded "catalytic core" sequence, a single-stranded loop or a single-stranded recognition sequence. These molecules are able to cleave (preferably, repeatedly cleave) separate  
10 RNA or DNA molecules in a nucleotide base sequence specific manner. Such molecules can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript. Such molecules also include nucleic acid molecules having a 3' or 5' non-nucleotide, useful as a capping group to prevent exonuclease digestion.

15 Non-nucleotide mimetics useful in this invention are generally described above and in Usman et al. WO 95/06731. Those in the art will recognize that these mimetics can be incorporated into an enzymatic molecule by standard techniques at any desired location. Suitable choices can be made by standard experiments to determine the best location, e.g., by synthesis of the  
20 molecule and testing of its enzymatic activity. The optimum molecule will contain the known ribonucleotides needed for enzymatic activity, and will have non-nucleotides which change the structure of the molecule in the least way possible. What is desired is that several nucleotides can be substituted by one non-nucleotide to save synthetic steps in enzymatic molecule synthesis  
25 and to provide enhanced stability of the molecule compared to RNA or even DNA.

### Synthesis

This invention relates to the synthesis, deprotection, and purification of enzymatic RNA or modified enzymatic RNA molecules in milligram to kilogram  
30 quantities with high biological activity. Such syntheses are generally detailed in Stinchcomb et al., WO 95/23225.

This invention relates to the synthesis, deprotection, and purification of enzymatic RNA or modified enzymatic RNA molecules in milligram to kilogram quantities with high biological activity.

Generally, RNA is synthesized and purified by methodologies based on:  
5   tetrazole to activate the RNA amidite,  $\text{NH}_4\text{OH}$  to remove the exocyclic amino protecting groups, tetra-*n*-butylammonium fluoride (TBAF) to remove the 2'-OH alkylsilyl protecting groups, and gel purification and analysis of the deprotected RNA. In particular this applies to, but is not limited to, a certain class of RNA molecules, ribozymes. These may be formed either chemically  
10   or using enzymatic methods. Examples of the chemical synthesis, deprotection, purification and analysis procedures are provided by Usman et al., 1987 *J. American Chem. Soc.*, 109, 7845, Scaringe et al. *Nucleic Acids Res.* 1990, 18, 5433-5341, Perreault et al. *Biochemistry* 1991, 30 4020-4025, and Slim and Gait *Nucleic Acids Res.* 1991, 19, 1183-1188. Odai et al. *FEBS Lett.* 1990, 267, 150-152 describes a reverse phase chromatographic purification of RNA fragments used to form a ribozyme. All the above noted  
15   references are all hereby incorporated by reference herein.

The aforementioned chemical synthesis, deprotection, purification and analysis procedures are time consuming (10-15 m coupling times) and may  
20   also be affected by inefficient activation of the RNA amidites by tetrazole, time consuming (6-24 h) and incomplete deprotection of the exocyclic amino protecting groups by  $\text{NH}_4\text{OH}$ , time consuming (6-24 h), incomplete and difficult to desalt TBAF-catalyzed removal of the alkylsilyl protecting groups, time consuming and low capacity purification of the RNA by gel  
25   electrophoresis, and low resolution analysis of the RNA by gel electrophoresis.

Imazawa and Eckstein, 1979 *J. Org. Chem.*, 12, 2039, describe the synthesis of 2'-amino-2'-deoxyribofuranosyl purines. They state that—

30    “To protect the 2'-amino function, we selected the trifluoroacetyl group which can easily be removed.”

### Chemical linkage

Jennings et al., US Patent No. 5,298,612 describe the use of non-nucleotides to assemble a hammerhead ribozyme lacking a stem II portion.

Draper et al., WO 93/23569 (PCT/US93/04020) describes synthesis of  
5 ribozymes in two parts in order to aid in the synthetic process (see, e.g., p. 40).

Usman et al., WO 95/06731, describe enzymatic nucleic acid molecules having non-nucleotides within their structure. Such non-nucleotides can be used in place of nucleotides to allow formation of an enzymatic nucleic acid.

This invention relates to improved methods for synthesis of enzymatic  
10 nucleic acids and, in particular, hammerhead and hairpin motif ribozymes. This invention is advantageous over iterative chemical synthesis of ribozymes since the yield of the final ribozyme can be significantly increased. Rather than synthesizing, for example, a 37mer hammerhead ribozyme, two partial ribozyme portions, e.g., a 20mer and a 17mer, can be synthesized in  
15 significantly higher yield, and the two reacted together to form the desired enzymatic nucleic acid.

Referring to Fig. 68, the strategy involved is shown for a hammerhead ribozyme where each n or n' is independently any desired nucleotide or non-nucleotide, each filled-in circle represents pairing between bases or other  
20 entities, and the solid line represents a covalent bond. Within the structure each n and n' may be a ribonucleotide, a 2'-methoxy-substituted nucleotide, or any other type of nucleotide which does not significantly affect the desired enzymatic activity of the final product (see Usman et al., supra). In the particular embodiment shown, which is not limiting in this invention, five  
25 ribonucleotides are provided at rG5, rA6, rG8, rG12, and rA15.1. U4 and U7 may be abasic (i.e., lacking the uridine moiety) or may be ribonucleotides, 2'-methoxy substituted nucleotides, or other such nucleotides. a9, a13, and a14 are preferably 2'-methoxy or may have other substituents. The synthesis of this hammerhead ribozyme is performed by synthesizing a 3' and a 5' portion  
30 as shown in a lower part of Fig. 68. Each 5' and 3' portion has a chemically reactive group X and Y, respectively. Non-limiting examples of such chemically reactive groups are provided in Fig. 69. These groups undergo



chemical reactions to provide the bonds shown in Fig. 69. Thus, the X and Y can be used, in various combinations, in this invention to form a chemical linkage between two ribozyme portions.

Thus, the invention features a method for synthesis of an enzymatically  
5 active nucleic acid (as defined by Draper, supra) by providing a 3' and a 5' portion of that nucleic acid, each having independently chemically reactive groups at the 5' and 3' positions, respectively. The reaction is performed under conditions in which a covalent bond is formed between the 3' and 5' portions by those chemically reactive groups. The bond formed can be, but is  
10 not limited to, either a disulfide, morpholino, amide, ether, thioether, amine, a double bond, a sulfonamide, carbonate, hydrazone or ester bond. The bond is not the natural bond formed between a 5' phosphate group and a 3' hydroxyl group which is made during normal synthesis of an oligonucleotide. In other embodiments, more than two portions can be linked together using  
15 pairs of X and Y groups which allow proper formation of the ribozyme (see Figure 69).

By "chemically reactive group" is simply meant a group which can react with another group to form the desired bonds. These bonds may be formed under any conditions which will not significantly affect the structure of the  
20 resulting enzymatic nucleic acid. Those in the art will recognize that suitable protecting groups can be provided on the ribozyme portions.

In preferred embodiments the nucleic acid has a hammerhead motif and the 3' and 5' portions each have chemically reactive groups in or immediately adjacent to the stem II region (see Fig. 1). The stem II region is evident in Fig.  
25 1 between the bases termed a9 and rG12. The C and G within this stem defines the end of the stem II region. Thus, any of the n or n' moieties within the stem II region can be provided with a chemically reactive group. As is evident from this structure, the chemically reactive groups need not be provided in the solid line portion but can be provided at any of the n or n'. In  
30 this way the length of each of the 5' and 3' portions can vary by several bases (Figure 70).

In other preferred embodiments, the chemically reactive group can be, but is not limited to,  $(\text{CH}_2)_n\text{SH}$ ;  $(\text{CH}_2)_n\text{NHR}$ ;  $(\text{CH}_2)_n\text{X}$ ; ribose;  $\text{COOH}$ ;  $(\text{CH}_2)_n\text{PPh}_3$ ;  $(\text{CH}_2)_n\text{SO}_2\text{Cl}$ ;  $(\text{CH}_2)_n\text{COR}$ ;  $(\text{CH}_2)_n\text{RNH}$  or  $(\text{CH}_2)_n\text{OH}$ , where,  $\text{CH}_2$  can be replaced by another group which forms a linking chain (which does not interfere with the terminal chemically reactive group) containing various atoms including, but not limited to  $\text{CH}_2$ , such as methylenes, ether, ethylene glycol, thioethers, double bonds, aromatic groups and others, generally at most 20 such atoms are provided in the linking chain, most preferably only 5 - 10 atoms, and even more preferably only 3- 5 atoms; each  $n$  independently is an integer from 0 to 10 inclusive and may be the same or different; each  $R$  independently is a proton or an alkyl, alkenyl (as described above) and other functional groups or conjugates such as peptides, steroids, hormones, lipids, nucleic acid sequences and others that provides nuclease resistance, improved cell association, improved cellular uptake or intercellular localization.  $X$  is halogen, and  $\text{Ph}$  represents a phenyl ring.

In yet other preferred embodiments, the conditions include provision of  $\text{NaIO}_4$  in contact with the ribose, and subsequent provision of a reducing group such as  $\text{NaBH}_4$  or  $\text{NaCNBH}_3$ ; or the conditions include provision of a coupling reagent.

In a second related aspect, the invention features a mixture of the 5' and 3' portions of the enzymatically active nucleic acids having the 3' and 5' chemically reactive groups noted above.

Those in the art will recognize that while examples are provided of half ribozymes it is possible to provide ribozymes in 3 or more portions. For example, the hairpin ribozyme may be synthesized by inclusion of chemically reactive groups in helix IV and in other helices which are not critical to the enzymatic activity of the nucleic acid.

#### Pol III-based vectors

This invention relates to RNA polymerase III-based methods and systems for expression of therapeutic RNAs in cells *in vivo* or *in vitro*.

The RNA polymerase III (pol III) promoter is one found in DNA encoding 5S, U6, adenovirus VA1, Vault, telomerase RNA, tRNA genes, etc., and is transcribed by RNA polymerase III (for a review see Geiduschek and Tocchini-Valentini, 1988 *Annu. Rev. Biochem.* 57, 873-914; Willis, 1993 *Eur. J. Biochem.* 212, 1-11). There are three major types of pol III promoters: types 1, 2 and 3 (Geiduschek and Tocchini-Valentini, 1988 *supra*; Willis, 1993 *supra*) (see Figure 1). Type 1 pol III promoter consists of three cis-acting sequence elements downstream of the transcriptional start site a) 5' sequence element (A block); b) an intermediate sequence element (I block); c) 3' sequence element (C block). 5S ribosomal RNA genes are transcribed using the type 1 pol III promoter (Specht et al., 1991 *Nucleic Acids Res.* 19, 2189-2191).

The type 2 pol III promoter is characterized by the presence of two cis-acting sequence elements downstream of the transcription start site. All Transfer RNA (tRNA), adenovirus VA RNA and Vault RNA (Kikhoef et al., 1993, *J. Biol. Chem.* 268, 7868-7873) genes are transcribed using this promoter (Geiduschek and Tocchini-Valentini, 1988 *supra*; Willis, 1993 *supra*). The sequence composition and orientation of the two cis-acting sequence elements- A box (5' sequence element) and B box (3' sequence element) are essential for optimal transcription by RNA polymerase III.

The type 3 pol III promoter contains all of the cis-acting promoter elements upstream of the transcription start site. Upstream sequence elements include a traditional TATA box (Mataj et al., 1988 *Cell* 55, 435-442), proximal sequence element (PSE) and a distal sequence element (DSE; Gupta and Reddy, 1991 *Nucleic Acids Res.* 19, 2073-2075). Examples of genes under the control of the type 3 pol III promoter are U6 small nuclear RNA (U6 snRNA) and Telomerase RNA genes.

In addition to the three predominant types of pol III promoters described above, several other pol III promoter elements have been reported (Willis, 1993 *supra*) (see Figure 76). Epstein-Barr-virus-encoded RNAs (EBER), *Xenopus* seleno-cysteine tRNA and human 7SL RNA are examples of genes that are under the control of pol III promoters distinct from the aforementioned types of promoters. EBER genes contain a functional A and B box (similar to type 2 pol III promoter). In addition they also require an EBER-specific TATA

box and binding sites for ATF transcription factors (Howe and Shu, 1989 *Cell* 57,825-834). The seleno-cysteine tRNA gene contains a TATA box, PSE and DSE (similar to type 3 pol III promoter). Unlike most tRNA genes, the seleno-cysteine tRNA gene lacks a functional A box sequence element. It does  
5 require a functional B box (Lee et al., 1989 *J. Biol. Chem.* 264, 9696-9702). The human 7SL RNA gene contains an unique sequence element downstream of the transcriptional start site. Additionally, upstream of the transcriptional start site, the 7SL gene contains binding sites for ATF class of transcription factors and a DSE (Bredow et al., 1989 *Gene* 86, 217-225).

10 Gilboa WO 89/11539 and Gilboa and Sullenger WO 90/13641 describe transformation of eucaryotic cells with DNA under the control of a pol III promoter. They state:

"In an attempt to improve antisense RNA synthesis using stable gene transfer protocols, the use of pol III promoters to drive the expression of antisense RNA can be  
15 considered. The underlying rationale for the use of pol III promoters is that they can generate substantially higher levels of RNA transcripts in cells as compared to pol II promoters. For example, it is estimated that in a eucaryotic cell there are about  $6 \times 10^7$  t-RNA molecules and  $7 \times 10^5$  mRNA molecules, i.e., about 100 fold more pol III transcripts of this class than total pol II transcripts. Since there are about 100 active t-RNA genes  
20 per cell, each t-RNA gene will generate on the average RNA transcripts equal in number to total pol II transcripts. Since an abundant pol II gene transcript represents about 1% of total mRNA while an average pol II transcript represents about 0.01% of total mRNA, a t-RNA (pol III) based transcriptional unit may be able to generate 100 fold to 10,000 fold more RNA than a pol II based transcriptional unit. Several reports have described the  
25 use of pol III promoters to express RNA in eucaryotic cells. Lewis and Manley and Sisodia have fused the Adenovirus VA-1 promoter to various DNA sequences (the herpes TK gene, globin and tubulin) and used transfection protocols to transfer the resulting DNA constructs into cultured cells which resulted in transient synthesis of RNA in the transduced cell. De la Pena and Zasloff have expressed a t-RNA-Herpes TK  
30 fusion DNA construct upon microinjection into frog oocytes. Jennings and Molloy have constructed an antisense RNA template by fusing the VA-1 gene promoter to a DNA fragment derived from SV40 based vector which also resulted in transient expression of antisense RNA and limited inhibition of the target gene". [Citations omitted.]

The authors describe a fusion product of a chimeric tRNA and an RNA product (see Fig. 1C of WO 90/13641). In particular they describe a human tRNA<sup>met</sup> derivative 3-5. 3-5 was derived from a cloned human tRNA gene by deleting 19 nucleotides from the 3' end of the gene. The authors indicate that  
5 the truncated gene can be transcribed if a termination signal is provided, but that no processing of the 3' end of the RNA transcript takes place.

Adeniyi-Jones et al., 1984 *Nucleic Acids Res.* 12, 1101-1115, describe certain constructions which "may serve as the basis for utilizing the tRNA gene as a 'portable promoter' in engineered genetic constructions." The authors  
10 describe the production of a so-called  $\Delta 3'-5$  in which 11 nucleotides of the 3'-end of the mature tRNA<sup>met</sup> sequence are replaced by a plasmid sequence, and are not processed to generate a mature tRNA. The authors state:

"the properties of the tRNA<sup>met</sup> 3' deletion plasmids described in this study suggest their potential use in certain engineered genetic constructions. The tRNA gene could  
15 be used to promote transcription of theoretically any DNA sequence fused to the 3' border of the gene, generating a fusion gene which would utilize the efficient polymerase III promoter of the human tRNA<sup>met</sup> gene. By fusion of the DNA sequence to a tRNA<sup>met</sup> deletion mutant such as  $\Delta 3'-4$ , a long read-through transcript would be generated in vivo (dependent, of course, on the absence of effective RNA polymerase  
20 III termination sequences). Fusion of the DNA sequence to a tRNA<sup>met</sup> deletion mutant such as  $\Delta 3'-5$  would lead to the generation of a co-transcript from which subsequent processing of the tRNA leader at the 5' portion of the fused transcript would be blocked. Control over processing may be of some biological use in engineered constructions, as suggested by properties of mRNA species bearing tRNA sequences as 5' leaders in  
25 prokaryotes. Such "dual transcripts" code for several predominant bacterial proteins such as EF-Tu and may use the tRNA leaders as a means of stabilizing the transcript from degradation in vivo. The potential use of the tRNA<sup>met</sup> gene as a "promoter leader" in eukaryotic systems has been realized recently in our laboratory. Fusion genes consisting of the deleted tRNA<sup>met</sup> sequences contained on plasmids  $\Delta 3'-4$   
30 and  $\Delta 3'-5$  in front of a promoter-less Herpes simplex type I thymidine kinase gene yield viral-specific enzyme resulting from RNA polymerase III dependent transcription in both *X. laevis* oocytes and somatic cells". [References omitted].

Sullenger et al., 1990 *Cell* 63, 601-619, describe over-expression of *TAR*-containing sequences using a chimeric *tRNA<sup>met</sup>-TAR* transcription unit in a double copy (DC) murine retroviral vector.

5 Sullenger et al., 1990 *Molecular and Cellular Bio.* 10, 6512, describe expression of chimeric tRNA driven antisense transcripts. It indicates:

10 "successful use of a tRNA-driven antisense RNA transcription system was dependent on the use of a particular type of retroviral vector, the double-copy (DC) vector, in which the chimeric tRNA gene was inserted in the viral LTR. The use of an RNA pol III-based transcription system to stably express high levels of foreign RNA sequences in cells may have other important applications. Foremost, it may significantly improve the ability to inhibit endogenous genes in eucaryotic cells for the study of gene expression and function, whether antisense RNA, ribozymes, or competitors of sequence-specific binding factors are used. tRNA-driven transcription systems may be particularly useful for introducing "mutations" into the germ line, i.e., for generating transgenic animals or transgenic plants. Since tRNA genes are ubiquitously expressed in all cell types, the chimeric tRNA genes may be properly expressed in all tissues of the animal, in contrast to the more idiosyncratic behavior of RNA pol II-based transcription units. However, homologous recombination represents a more elegant although, at present, very cumbersome approach for introducing mutations into the germ line. In either case, the ability to generate transgenic animals or plants carrying defined mutations will be an extremely valuable experimental tool for studying gene function in a developmental context and for generating animal models for human genetic disorders. In addition, tRNA-driven gene inhibition strategies may also be useful in creating pathogen-resistant livestock and plants. [References omitted.]

25 Cotten and Bimstiel, 1989 *EMBO Jml.* 8, 3861, describe the use of tRNA genes to increase intracellular levels of ribozymes. The authors indicate that the ribozyme coding sequences were placed between the A and the B box internal promoter sequences of the *Xenopus tRNA<sup>met</sup>* gene. They also indicate that the targeted hammerhead ribozymes were active *in vivo*.

30 Yu et al., 1993 *Proc. Natl. Acad. Sci. USA* 90, 5340, describe the use of a VAI promoter to express a hairpin ribozyme. The resulting transcript consisted

of the first 104 nucleotides of the VAI RNA, followed by the ribozyme sequence and the terminator sequence.

Lieber and Strauss, 1995 *Mol. Cellular Bio.* 15, 540, inserted a hammerhead ribozyme sequence in the central domain of a VAI RNA.

- 5 Pol III-based vectors are described in Stinchcomb et al., WO 95/23225. Another example is provided below.

Example 1: Stromelysin Hammerhead ribozymes

- By engineering ribozyme motifs applicant has designed several ribozymes directed against stromelysin mRNA sequences. These ribozymes  
10 are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave stromelysin target sequences *in vitro* is evaluated.

- The ribozymes are tested for function *in vivo* by analyzing stromelysin expression levels. Ribozymes are delivered to cells by incorporation into  
15 liposomes, by complexing with cationic lipids, by microinjection, and/or by expression from DNA/RNA vectors. Stromelysin expression is monitored by biological assays, ELISA, by indirect immunofluorescence, and/or by FACS analysis. Stromelysin mRNA levels are assessed by Northern analysis, RNase protection, primer extension analysis and/or quantitative RT-PCR.  
20 Ribozymes that block the induction of stromelysin activity and/or stromelysin mRNA by more than 50% are identified.

- Ribozymes targeting selected regions of mRNA associated with arthritic disease are chosen to cleave the target RNA in a manner which preferably inhibits translation of the RNA. Genes are selected such that inhibition of  
25 translation will preferably inhibit cell replication, e.g., by inhibiting production of a necessary protein or prevent production of an undesired protein, e.g., stromelysin. Selection of effective target sites within these critical regions of mRNA may entail testing the accessibility of the target RNA to hybridization with various oligonucleotide probes. These studies can be performed using  
30 RNA or DNA probes and assaying accessibility by cleaving the hybrid molecule with RNaseH (see below). Alternatively, such a study can use

ribozyme probes designed from secondary structure predictions of the mRNAs, and assaying cleavage products by polyacrylamide gel electrophoresis (PAGE), to detect the presence of cleaved and uncleaved molecules.

5 In addition, potential ribozyme target sites within the rabbit stromelysin mRNA sequence (1795 nucleotides) were located and aligned with the human target sites. Because the rabbit stromelysin mRNA sequence has an 84% sequence identity with the human sequence, many ribozyme target sites are also homologous. Thus, the rabbit has potential as an appropriate animal  
10 model in which to test ribozymes that are targeted to human stromelysin but have homologous or nearly homologous cleavage sites on rabbit stromelysin mRNA as well (Tables AII-AVI, AVIII & AIX ). Thirty of the 316 UH sites in the rabbit sequence are identical with the corresponding site in the human sequence with respect to at least 14 nucleotides surrounding the potential  
15 ribozyme cleavage sites. The nucleotide in the RNA substrate that is immediately adjacent (5') to the cleavage site is unpaired in the ribozyme-substrate complex (see Fig. 1) and is consequently not included in the comparison of human and rabbit potential ribozyme sites. In choosing human ribozyme target sites for continued testing, the presence of identical or nearly  
20 identical sites in the rabbit sequence is considered.

#### Example 2: Superior sites

Potential ribozyme target sites were subjected to further analysis using computer folding programs (Mfold or a Macintosh-based version of the following program, LRNA (Zucker (1989) Science 244:48), to determine if 1)  
25 the target site is substantially single-stranded and therefore predicted to be available for interaction with a ribozyme, 2) if a ribozyme designed to that site is predicted to form stem II but is generally devoid of any other intramolecular base pairing, and 3) if the potential ribozyme and the sequence flanking both sides of the cleavage site together are predicted to interact correctly. The  
30 sequence of Stem II can be altered to maintain a stem at that position but minimize intramolecular basepairing with the ribozyme's substrate binding arms. Based on these minimal criteria, and including all the sites that are identical in human and rabbit stromelysin mRNA sequence, a subset of 66



potential superior ribozyme target sites was chosen (as first round targets) for continued analysis. These are SEQ. ID. NOS.: 34, 35, 37, 47, 54, 57, 61, 63, 64, 66, 76, 77, 79, 87, 88, 96, 97, 98, 99, 100, 107, 110, 121, 126, 128, 129, 133, 140, 146, 148, 151, 162, 170, 179, 188, 192, 194, 196, 199, 202, 203, 207, 208, 218, 220, 223, 224, 225, 227, 230, 232, 236, 240, 245, 246, 256, 259, 260, 269, 280, 281, 290, 302, 328, 335 and 353 (see Table AIII).

### Example 3: Accessible sites

To determine if any or all of these potential superior sites might be accessible to a ribozyme directed to that site, an RNase H assay is carried out. Using this assay, the accessibility of a potential ribozyme target site to a DNA oligonucleotide probe can be assessed without having to synthesize a ribozyme to that particular site. If the complementary DNA oligonucleotide is able to hybridize to the potential ribozyme target site then RNase H, which has the ability to cleave the RNA of a DNA/RNA hybrid, will be able to cleave the target RNA at that particular site. Specific cleavage of the target RNA by RNase H is an indication that that site is "open" or "accessible" to oligonucleotide binding and thus predicts that the site will also be open for ribozyme binding. By comparing the relative amount of specific RNase H cleavage products that are generated for each DNA oligonucleotide/site, potential ribozyme sites can be ranked according to accessibility.

To analyze target sites using the RNase H assay, DNA oligonucleotides (generally 13-15 nucleotides in length) that are complementary to the potential target sites are synthesized. Body-labeled substrate RNAs (either full-length RNAs or ~500-600 nucleotide subfragments of the entire RNA) are prepared by *in vitro* transcription in the presence of a <sup>32</sup>P-labeled nucleotide. Unincorporated nucleotides are removed from the <sup>32</sup>P-labeled substrate RNA by spin chromatography on a G-50 Sephadex column and used without further purification. To carry out the assay, the <sup>32</sup>P-labeled substrate RNA is pre-incubated with the specific DNA oligonucleotide (1 μM and 0.1 μM final concentration) in 20 mM Tris-HCl, pH 7.9, 100 mM KCl, 10 mM MgCl<sub>2</sub>, 0.1 mM EDTA, 0.1 mM DTT at 37°C for 5 minutes. An excess of RNase H (0.8 units/10 μl reaction) is added and the incubation is continued for 10 minutes. The reaction is quenched by the addition of an equal volume of 95% formamide.

20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol FF after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. RNase H-cleaved RNA products are separated from uncleaved RNA on denaturing polyacrylamide gels, visualized by autoradiography and the amount of cleavage product is quantified.

RNase H analysis on the 66 potential ribozyme sites (round 1) was carried out and those DNA oligonucleotides/sites that supported the most RNase H cleavage were determined. These assays were carried out using full-length human and rabbit stromelysin RNA as substrates. Results determined on human stromelysin RNA indicated that 23 of the 66 sites supported a high level of RNase H cleavage, and an additional 13 supported a moderate level of RNase H cleavage. Twenty-two sites were chosen from among these two groups for continued study. Two of the criteria used for making this choice were 1) that the particular site supported at least moderate RNase H cleavage on human stromelysin RNA and 2) that the site have two or fewer nucleotide differences between the rabbit and the human stromelysin sequence. RNase H accessibility on rabbit stromelysin RNA was determined, but was not used as a specific criteria for these choices. Those DNA oligonucleotides that are not totally complementary to the rabbit sequence may not be good indicators of the relative amount of RNase H cleavage, possibly because the mismatch leads to less efficient hybridization of the DNA oligonucleotide to the mismatched RNA substrate and therefore less RNase H cleavage is seen.

#### Example 4: Analysis of Ribozymes

Ribozymes were then synthesized to 22 sites (Table AV) predicted to be accessible as judged the RNase H assay. Eleven of these 22 sites are identical to the corresponding rabbit sites. The 22 sites are SEQ. ID, NOS.: 34, 35, 57, 125, 126, 127, 128, 129, 140, 162, 170, 179, 188, 223, 224, 236, 245, 246, 256, 259, 260, 281. The 22 ribozymes were chemically synthesized with recognition arms of either 7 nucleotides or 8 nucleotides, depending on which ribozyme alone and ribozyme-substrate combinations were predicted by the computer folding program (Mfold) to fold most correctly. After synthesis, ribozymes are either purified by HPLC or gel purified.

These 22 ribozymes were then tested for their ability to cleave both human and rabbit full-length stromelysin RNA. Full-length, body-labeled stromelysin RNA is prepared by *in vitro* transcription in the presence of [ $\alpha$ - $^{32}$ P]CTP, passed over a G 50 Sephadex column by spin chromatography and used as substrate RNA without further purification. Assays are performed by prewarming a 2X concentration of purified ribozyme in ribozyme cleavage buffer (50 mM Tris-HCl, pH 7.5 at 37°C, 10 mM MgCl<sub>2</sub>) and the cleavage reaction is initiated by adding the 2X ribozyme mix to an equal volume of substrate RNA (maximum of 1-5 nM) that has also been prewarmed in cleavage buffer. As an initial screen, assays are carried out for 1 hour at 37°C using a final concentration of 1  $\mu$ M and 0.1  $\mu$ M ribozyme, *i.e.*, ribozyme excess. The reaction is quenched by the addition of an equal volume of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol FF after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. Full-length substrate RNA and the specific RNA products generated by ribozyme cleavage are visualized on an autoradiograph of the gel.

Of the 22 ribozymes tested, 21 were able to cleave human and rabbit substrate RNA *in vitro* in a site-specific manner. In all cases, RNA cleavage products of the appropriate lengths were visualized. The size of the RNA was judged by comparison to molecular weight standards electrophoresed in adjacent lanes of the gel. The fraction of substrate RNA cleaved during a ribozyme reaction can be used as an assessment of the activity of that ribozyme *in vitro*. The activity of these 22 ribozymes on full-length substrate RNA ranged from approximately 10% to greater than 95% of the substrate RNA cleaved in the ribozyme cleavage assay using 1  $\mu$ M ribozyme as described above. A subset of seven of these ribozymes was chosen for continued study. These seven ribozymes (denoted in Table AV) were among those with the highest activity on both human and rabbit stromelysin RNA. Five of these seven sites have sequence identity between human and rabbit stromelysin RNAs for a minimum of 7 nucleotides in both directions flanking the cleavage site. These sites are 883, 947, 1132, 1221 and 1410. and the ribozymes are SEQ. ID. NOS.: 368, 369, 370, 371, 372, 373, and 374.

Example 5: Arm Length Tests

In order to test the effect of arm length variations on the cleavage activity of a ribozyme to a particular site *in vitro*, ribozymes to these seven sites were designed that had alterations in the binding arm lengths. For each site, a complete set of ribozymes was synthesized that included ribozymes with binding arms of 6 nucleotides, 7 nucleotides, 8 nucleotides, 10 nucleotides and 12 nucleotides, i.e., 5 ribozymes to each site. These ribozymes were gel-purified after synthesis and tested in ribozyme cleavage assays as described above.

After analysis of the 35 ribozymes, five ribozymes with varied arm lengths to each of these seven sites, it was clear that two ribozymes were the most active *in vitro*. These two ribozymes had seven nucleotide arms directed against human sequence cleavage sites of nucleotide 617 and nucleotide 820. These are referred to as RZ 617H 7/7 and RZ 820H 7/7 denoting the human (H) sequence cleavage site (617 or 820) and the arm length on the 5' and 3' side of the ribozyme molecule.

Example: Testing the efficacy of ribozymes in cell culture

The two most active ribozymes *in vitro* (RZ 617H 7/7 and RZ 820H 7/7) were then tested for their ability to cleave stromelysin mRNA in the cell. Primary cultures of human or rabbit synovial fibroblasts were used in these experiments. For these efficacy tests, ribozymes with 7 nucleotide arms were synthesized with 2' O- methyl modifications on the 5 nucleotides at the 5' end of the molecule and on the 5 nucleotides at the 3' end of the molecule. For comparison, ribozymes to the same sites but with 12 nucleotide arms (RZ 617H 12/12 and RZ 820H 12/12) were also synthesized with the 2' O methyl modifications at the 5 positions at the end of both binding arms. Inactive ribozymes that contain 2 nucleotide changes in the catalytic core region were also prepared for use as controls. The catalytic core in the inactive ribozymes is C U U A U G A G G C C G A A A G G C C G A U versus C U G A U G A G G C C G A A A G G C C G A A in the active ribozymes. The inactive ribozymes show no cleavage activity *in vitro* when measured on full-length RNA in the typical ribozyme cleavage assay at a 1  $\mu$ M concentration for 1 hour.

The general assay was as follows: Fibroblasts, which produce stromelysin, are serum-starved overnight and ribozymes or controls are offered to the cells the next day. Cells are maintained in serum-free media. The ribozyme can be applied to the cells as free ribozyme, or in association with various delivery vehicles such as cationic lipids (including Transfectam™, Lipofectin™ and Lipofectamine™), conventional liposomes, non-phospholipid liposomes or biodegradable polymers. At the time of ribozyme addition, or up to 3 hours later, Interleukin-1 $\alpha$  (typically 20 units/ml) can be added to the cells to induce a large increase in stromelysin expression. The production of stromelysin can then be monitored over a time course, usually up to 24 hours.

If a ribozyme is effective in cleaving stromelysin mRNA within a cell, the amount of stromelysin mRNA will be decreased or eliminated. A decrease in the level of cellular stromelysin mRNA, as well as the appearance of the RNA products generated by ribozyme cleavage of the full-length stromelysin mRNA, can be analyzed by methods such as Northern blot analysis, RNase protection assays and/or primer extension assays. The effect of ribozyme cleavage of cellular stromelysin mRNA on the production of the stromelysin protein can also be measured by a number of assays. These include the ELISA (Enzyme-Linked Immuno Sorbent Assay) and an immunofluorescence assay described below. In addition, functional assays have been published that monitor stromelysin's enzymatic activity by measuring degradation of its primary substrate, proteoglycan.

#### Example 7: Analysis of Stromelysin Protein

Stromelysin secreted into the media of Interleukin-1 $\alpha$ -induced human synovial fibroblasts was measured by ELISA using an antibody that recognizes human stromelysin. Where present, a Transfectam™-ribozyme complex (0.15  $\mu$ M ribozyme final concentration) was offered to  $2-4 \times 10^5$  serum-starved cells for 3 hours prior to induction with Interleukin-1 $\alpha$ . The Transfectam™ was prepared according to the manufacturer (Promega Corp.) except that 1:1 (w/w) dioleoyl phosphatidylethanolamine was included. The Transfectam™-ribozyme complex was prepared in a 5:1 charge ratio. Media was harvested 24 hours after the addition of Interleukin-1 $\alpha$ . The control (NO RZ) is Transfectam™ alone applied to the cell. Inactive ribozymes, with 7

nucleotide arms or 12 nucleotide arms have the two inactivating changes to the catalytic core that are described above. Cell samples were prepared in duplicate and the assay was carried out on several dilutions of the conditioned media from each sample. Results of the ELISA are presented below as a percent of stromelysin present vs. the control (NO RZ) which is set at 100%.

		<u>RZ TARGET SITE</u>	
<u>TREATMENT</u>		<u>617H</u>	<u>820H</u>
	RZ 7/7	06.83	07.05
	RZ 12/12	18.47	33.90
10	INACTIVE RZ 7/7	100	100
	INACTIVE RZ 12/12	100	100
	NO RZ CONTROL	100	100

15 The results above clearly indicate that treatment with active ribozyme, either RZ 617H 7/7 and RZ 820H 7/7, has a dramatic effect on the amount of stromelysin secreted by the cells. When compared to untreated, control cells or cells treated with inactive ribozymes, the level of stromelysin was decreased by approximately 93%. Ribozymes to the same sites, but  
 20 synthesized with 12 nucleotide binding arms, were also efficacious, causing a decrease in stromelysin to ~66 to ~81% of the control. In previous *in vitro* ribozyme cleavage assays, RZ 617H 7/7 and RZ 820H 7/7 had better cleavage activity on full-length RNA substrates than ribozymes with 12 nucleotide arms directed to the same sites (617H 12/12 and RZ 820H 12/12).

### 25 Example 8: Immunofluorescent Assay

An alternative method of stromelysin detection is to visualize stromelysin protein in the cells by immunofluorescence. For this assay, cells are treated

with monensin to prevent protein secretion from the cell. The stromelysin retained by the cells after monensin addition can then be visualized by immunofluorescence using either conventional or confocal microscopy. Generally, cells were serum-starved overnight and treated with ribozyme the following day for several hours. Monensin was then added and after ~5-6 hours, monensin-treated cells were fixed and permeabilized by standard methods and incubated with an antibody recognizing human stromelysin. Following an additional incubation period with a secondary antibody that is conjugated to a fluorophore, the cells were observed by microscopy. A decrease in the amount of fluorescence in ribozyme-treated cells, compared to cells treated with inactive ribozymes or media alone, indicates that the level of stromelysin protein has been decreased due to ribozyme treatment.

As visualized by the immunofluorescence technique described above, treatment of human synovial fibroblasts with either RZ 617H 7/7 or RZ 820H 7/7 (final concentrations of 1.5  $\mu$ M free ribozyme or 0.15  $\mu$ M ribozyme complexed with Transfectam™ resulted in a significant decrease in fluorescence, and therefore stromelysin protein, when compared with controls. Controls consisted of treating with media or Transfectam™ alone. Treatment of the cells with the corresponding inactive ribozymes with two inactivating changes in the catalytic core resulted in immunofluorescence similar to the controls without ribozyme treatment.

Rabbit synovial fibroblasts were also treated with RZ 617H 7/7 or RZ 820H 7/7, as well as with the two corresponding ribozymes (RZ 617R 7/7 or RZ 820R 7/7) that each have the appropriate one nucleotide change to make them completely complementary to the rabbit target sequence. Relative to controls that had no ribozyme treatment, immunofluorescence in Interleukin-1 $\alpha$ -induced rabbit synovial fibroblasts was visibly decreased by treatment with these four ribozymes, whether specific for rabbit or human mRNA sequence. For the immunofluorescence study in rabbit synovial fibroblasts, the antibody to human stromelysin was used.

#### Example 9: Ribozyme Cleavage of Cellular RNA

The following method was used in this example.

Primer extension assay:

The primer extension assay was used to detect full-length RNA as well as the 3' ribozyme cleavage products of the RNA of interest. The method involves synthesizing a DNA primer (generally ~20 nucleotides in length) that  
5 can hybridize to a position on the RNA that is downstream (3') of the putative ribozyme cleavage site. Before use, the primer was labeled at the 5' end with  $^{32}\text{P}$ [ATP] using T4 polynucleotide kinase and purified from a gel. The labeled primer was then incubated with a population of nucleic acid isolated from a cellular lysate by standard procedures. The reaction buffer was 50 mM Tris-HCl, pH 8.3, 3 mM  $\text{MgCl}_2$ , 20 mM KCl, and 10 mM DTT. A 30 minute  
10 extension reaction follows, in which all DNA primers that have hybridized to the RNA were substrates for reverse transcriptase, an enzyme that will add nucleotides to the 3' end of the DNA primer using the RNA as a template. Reverse transcriptase was obtained from Life Technologies and is used  
15 essentially as suggested by the manufacturer. Optimally, reverse transcriptase will extend the DNA primer, forming cDNA, until the end of the RNA substrate is reached. Thus, for ribozyme-cleaved RNA substrates, the cDNA product will be shorter than the resulting cDNA product of a full-length, or uncleaved RNA substrate. The differences in size of the  $^{32}\text{P}$ -labeled  
20 cDNAs produced by extension can then be discriminated by electrophoresis on a denaturing polyacrylamide gel and visualized by autoradiography.

Strong secondary structure in the RNA substrate can, however, lead to premature stops by reverse transcriptase. This background of shorter cDNAs is generally not a problem unless one of these prematurely terminated  
25 products electrophoreses in the expected position of the ribozyme-cleavage product of interest. Thus, 3' cleavage products are easily identified based on their expected size and their absence from control lanes. Strong stops due to secondary structure in the RNA do, however, cause problems in trying to quantify the total full-length and cleaved RNA present. For this reason, only  
30 the relative amount of cleavage can easily be determined.

The primer extension assay was carried out on RNA isolated from cells that had been treated with Transfectam<sup>TM</sup>-complexed RZ 617H 7/7, RZ 820H 7/7, RZ 617H 12/12 and RZ 820H 12/12. Control cells had been treated with



Transfectam™ alone. Primer extensions on RNA from cells treated with the Transfectam™ complexes of the inactive versions of these four ribozymes were also prepared. The 20 nucleotide primer sequence is 5' AATGAAAACGAGGTCCTTGC 3' and it is complementary to a region about 5 285 nucleotides downstream of ribozyme site 820. For ribozymes to site 617, the cDNA length for the 3' cleavage product is 488 nucleotides, for 820 the cDNA product is 285 nucleotides. Full-length cDNA will be 1105 nucleotides in length. Where present, 1 ml of 0.15 µM ribozyme was offered to ~2-3 x 10<sup>5</sup> serum-starved human synovial fibroblasts. After 3 hours, 20 units/ml 10 Interleukin-1α was added to the cells and the incubation continued for 24 hours.

<sup>32</sup>P-labeled cDNAs of the correct sizes for the 3' products were clearly visible in lanes that contained RNA from cells that had been treated with active ribozymes to sites 617 and 820. Ribozymes with 7 nucleotide arms were 15 judged to be more active than ribozymes with 12 nucleotide arms by comparison of the relative amount of 3' cleavage product visible. This correlates well with the data obtained by ELISA analysis of the conditioned media from these same samples: In addition, no cDNAs corresponding to the 3' cleavage products were visible following treatment of the cells with any of 20 the inactive ribozymes.

To insure that ribozyme cleavage of the RNA substrate was not occurring during the preparation of the cellular RNA or during the primer extension reaction itself, several controls have been carried out. One control was to add body-labeled stromelysin RNA, prepared by *in vitro* transcription, to the 25 cellular lysate. This lysate was then subjected to the typical RNA preparation and primer extension analysis except that non-radioactive primer was used. If ribozymes that are present in the cell at the time of cell lysis are active under any of the conditions during the subsequent analysis, the added, body-labeled stromelysin RNA will become cleaved. This, however, is not the case. Only 30 full-length RNA was visible by gel analysis, no ribozyme cleavage products were present. This is evidence that the cleavage products detected in RNA from ribozyme-treated cells resulted from ribozyme cleavage in the cell, and not during the subsequent analysis.

### Example 10: RNase Protection Assay

By RNase protection analysis, both the 3' and the 5' products generated by ribozyme cleavage of the substrate RNA in a cell can be identified. The RNase protection assay is carried out essentially as described in the protocol provided with the Lysate Ribonuclease Protection Kit (United States Biochemical Corp.) The probe for RNase protection is an RNA that is complementary to the sequence surrounding the ribozyme cleavage site. This "antisense" probe RNA is transcribed *in vitro* from a template prepared by the polymerase chain reaction in which the 5' primer was a DNA oligonucleotide containing the T7 promoter sequence. The probe RNA is body labeled during transcription by including  $^{32}\text{P}$ [CTP] in the reaction and purified away from unincorporated nucleotide triphosphates by chromatography on G-50 Sephadex. The probe RNA (100,000 to 250,000 cpm) is allowed to hybridize overnight at 37°C to the RNA from a cellular lysate or to RNA purified from a cell lysate. After hybridization, RNase T<sub>1</sub> and RNase A are added to degrade all single-stranded RNA and the resulting products are analyzed by gel electrophoresis and autoradiography. By this analysis, full-length, uncleaved target RNA will protect the full-length probe. For ribozyme-cleaved target RNAs, only a portion of the probe will be protected from RNase digestion because the cleavage event has occurred in the region to which the probe binds. This results in two protected probe fragments whose size reflects the position at which ribozyme cleavage occurs and whose sizes add up to the size of the full-length protected probe.

RNase protection analysis was carried out on cellular RNA isolated from rabbit synovial fibroblasts that had been treated either with active or inactive ribozyme. The ribozymes tested had 7 nucleotide arms specific to the rabbit sequence but corresponding to human ribozyme sites 617 and 820 (i.e. RZ 617R 7/7, RZ 820R 7/7). The inactive ribozymes to the same sites also had 7 nucleotide arms and included the two inactivating changes described above. The inactive ribozymes were not active on full-length rabbit stromelysin RNA in a typical 1 hour ribozyme cleavage reaction *in vitro* at a concentration of 1  $\mu\text{M}$ . For all samples, one ml of 0.15  $\mu\text{M}$  ribozyme was administered as a Transfectam™ complex to serum-starved cells. Addition of Interleukin-1 $\alpha$  followed 3 hours later and cells were harvested after 24 hours. For samples

from cells treated with either active ribozyme tested, the appropriately-sized probe fragments representing ribozyme cleavage products were visible. For site 617, two fragments corresponding to 125 and 297 nucleotides were present, for site 820 the two fragments were 328 and 94 nucleotides in length.

- 5 No protected probe fragments representing RNA cleavage products were visible in RNA samples from cells that not been treated with any ribozyme, or in cells that had received the inactive ribozymes. Full-length protected probe (422 nucleotides in length) was however visible, indicating the presence of full-length, uncleaved stromelysin RNA in these samples.

10 Delivery of Free and Transfectam-Complexed Ribozymes to Fibroblasts

- Ribozymes can be delivered to fibroblasts complexed to a cationic lipid or in free form. To deliver free ribozyme, an appropriate dilution of stock ribozyme (final concentration is usually 1.5  $\mu$ M) is made in serum-free medium; if a radioactive tracer is to be used (i.e.,  $^{32}$ P), the specific activity of the ribozyme is adjusted to 800-1200 cpm/pmol. To deliver ribozyme complexed with the cationic lipid Transfectam, the lipid is first prepared as a stock solution containing 1/1 (w/w) dioleoylphosphatidylcholine (DOPE). Ribozyme is mixed with the Transfectam/DOPE mixture at a 1/5 (RZ/TF) charge ratio; for a 36-mer ribozyme, this is a 45-fold molar excess of Transfectam (Transfectam has 4 positive charges per molecule). After a 10 min incubation at room temperature, the mixture is diluted and applied to cells, generally at a ribozyme concentration of 0.15  $\mu$ M. For  $^{32}$ P experiments, the specific activity of the ribozyme is the same as for the free ribozyme experiments.

- After 24 hour, about 30% of the offered Transfectam-ribozyme cpm's are cell-associated (in a nuclease-resistant manner). Of this, about 10-15% of the cpm's represent intact ribozyme; this is about 20-25 million ribozymes per cell. For the free ribozyme, about 0.6% of the offered dose is cell-associated after 24 hours. Of this, about 10-15% is intact; this is about 0.6-0.8 million ribozymes per cell.

30 Example 11: *In vitro* cleavage of stromelysin mRNA by HH ribozymes

In order to screen for additional HH ribozyme cleavage sites, ribozymes, targeted against some of the sites listed in example 2 and Table 3, were

synthesized. These ribozymes were extensively modified such that: 5' terminal nucleotides contain phosphorothioate substitutions; except for five ribose residues in the catalytic core, all the other 2'-hydroxyl groups within the ribozyme were substituted with either 2'-O-methyl groups or 2'-C-allyl modifications. The aforementioned modifications are meant to be non-limiting modifications. Those skilled in the art will recognize that other embodiments can be readily generated using the techniques known in the art.

These ribozymes were tested for their ability to cleave RNA substrates *in vitro*. Referring to Fig. 7, *in vitro* RNA cleavage by HH ribozymes targeted to sites 21, 463, 1049, 1366, 1403, 1410 and 1489 (SEQ. ID. NOS. 35, 98, 202, 263, 279, 281 and 292 respectively) was assayed at 37°C. Substrate RNAs were 5' end-labeled using [ $\gamma$ -<sup>32</sup>P]ATP and T4 polynucleotide kinase enzyme. In a standard cleavage reaction under "ribozyme excess" conditions, ~1 nM substrate RNA and 40 nM ribozyme were denatured separately by heating to 90°C for 2 min followed by snap cooling on ice for 10 min. The substrate and the ribozyme reaction mixtures were renatured in a buffer containing 50 mM Tris-HCl, pH 7.5 and 10 mM MgCl<sub>2</sub> at 37°C for 10 min. Cleavage reaction was initiated by mixing the ribozyme and the substrate RNA and incubating at 37°C. Aliquots of 5  $\mu$ l were taken at regular intervals of time and the reaction quenched by mixing with an equal volume of formamide stop mix. The samples were resolved on a 20% polyacrylamide/urea gel.

A plot of percent RNA substrate cleaved as a function of time is shown in Fig. 7. The plot shows that all six HH ribozymes cleaved the target RNA efficiently. Some HH ribozymes were, however, more efficient than others (e.g., 1049HH cleaves faster than 1366HH).

#### Ribozyme Efficacy Assay in Cultured HS-27 Cells (Used in the Following Examples):

Ribozymes were assayed on either human foreskin fibroblasts(HS-27) cell line or primary human synovial fibroblasts (HSF). All cells were plated the day before the assay in media containing 10% fetal bovine serum in 24 well plates at a density of  $5 \times 10^4$  cells/well. At 24 hours after plating, the media was removed from the wells and the monolayers were washed with Dulbeccos

phosphate buffered saline (PBS). The cells were serum starved for 24 h by incubating the cells in media containing 0.5% fetal bovine serum (FBS; 1 ml/well). Ribozyme/lipid complexes were prepared as follows: Ribozymes and LipofectAMINE were diluted separately in serum-free DMEM plus 20 mM Hepes pH 7.3 to 2X final concentration, then equal volumes were combined, vortexed and incubated at 37°C for 15 minutes. The charge ratio of LipofectAmine: ribozyme was 3:1. Cells were washed twice with PBS containing  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . Cells were then treated the ribozyme/lipid complexes and incubated at 37°C for 1.5 hours. FBS was then added to a final concentration of 10%. Two hours after FBS addition, the ribozyme containing solution was removed and 0.5 ml DMEM containing 50 u/ml IL-1, 10% FBS, 20 mM Hepes pH 7.3 added. Supernatants were harvested 16 hours after IL-1 induction and assayed for stromelysin expression by ELISA. Polyclonal antibody against Matrix Metalloproteinase 3 (Biogenesis, NH) was used as the detecting antibody and anti-stromelysin monoclonal antibody was used as the capturing antibody in the sandwich ELISA (Maniatis et al., *supra*) to measure stromelysin expression.

Example 12: Ribozyme-Mediated Inhibition of Stromelysin Expression in human fibroblast cells

Referring to Figs. 8 through 13, HH ribozymes, targeted to sites 21, 463, 1049, 1366, 1403, 1410 and 1489 within human stromelysin-1 mRNA, were transfected into HS-27 fibroblast or HSF cell line as described above. Catalytically inactive ribozymes that contain 2 nucleotide changes in the catalytic core region were also synthesized for use as controls. The catalytic core in the inactive ribozymes was CUUAUGAGGCCGAAAGGCCGAU versus CUGAUGAGGCCGAAAGGCCGAA in the active ribozymes. The inactive ribozymes show no cleavage activity *in vitro* when measured on full-length RNA in the typical ribozyme cleavage assay at a 1  $\mu\text{M}$  concentration for 1 hour. Levels of stromelysin protein were measured using a sensitive ELISA protocol as described above. + IL-1 in the figures mean that cells were treated with IL-1 to induce the expression of stromelysin expression. -IL-1 means that the cells were not treated. Figs. 8 through 13 show the dramatic reduction in the levels of stromelysin protein expressed in cells that were transfected with active HH ribozymes. This decrease in the level of

stromelysin production is over and above some non-specific inhibition seen in cells that were transfected with catalytically inactive ribozymes. There is on an average a greater than 50% inhibition in stromelysin production (in cells transfected with active HH ribozymes) when compared with control cells that were transfected with inactive ribozymes. These results suggest that the reduction in stromelysin production in HS-27 cells is mediated by sequence-specific cleavage of human stromelysin-1 mRNA by catalytically active HH ribozymes. Reduction in stromelysin protein production in cells transfected with catalytically inactive ribozymes may be due to some "antisense effect" caused by binding of the inactive ribozyme to the target RNA and physically preventing translation.

Example 13: Ribozyme-mediated inhibition of stromelysin expression in Rabbit Knee

In order to extend the ribozyme efficacy in cell culture, applicant has chosen to use rabbit knee as a reasonable animal model to study ribozyme-mediated inhibition of rabbit stromelysin protein expression. Applicant selected a HH ribozyme (1049HH), targeted to site 1049 within human stromelysin-1 mRNA, for animal studies because site 1049 is 100% identical to site 1060 (Tables AIII and AVI) within rabbit stromelysin mRNA. This has enabled applicant to compare the efficacy of the same ribozyme in human as well as in rabbit systems.

Male New Zealand White Rabbits (3-4 Kg) were anaesthetized with ketamine-HCl/xylazine and injected intra-articularly (I.T.) in both knees with 100 µg ribozyme (e.g., SEQ. ID. NO. 202) in 0.5 ml phosphate buffered saline (PBS) or PBS alone (Controls). The IL-1 (human recombinant IL-1 $\alpha$ , 25 ng) was administered I.T., 24 hours following the ribozyme administration. Each rabbit received IL-1 in one knee and PBS alone in the other. The synovium was harvested 6 hours post IL-1 infusion, snap frozen in liquid nitrogen, and stored at -80°C. Total RNA is extracted with TRizol reagent (GIBCO BRL, Gaithersburg, MD), and was analyzed by Northern-blot analysis and/or RNase-protection assay. Briefly, 0.5 µg cellular RNA was separated on 1.0 % agarose/formaldehyde gel and transferred to Zeta-Probe GT nylon membrane (Bio-Rad, Hercules, CA) by capillary transfer for ~16 hours. The blots were

baked for two hours and then pre-hybridized for 2 hours at 65°C in 10 ml Church hybridization buffer (7 % SDS, 500 mM phosphate, 1 mM EDTA, 1% Bovine Serum Albumin). The blots were hybridized at 65°C for ~16 hours with  $10^6$  cpm/ml of full length  $^{32}\text{P}$ -labeled complementary RNA (cRNA) probes to rabbit stromelysin mRNA (cRNA added to the pre-hybridization buffer along with 100  $\mu\text{l}$  10mg/ml salmon sperm DNA). The blot was rinsed once with 5% SDS, 25 mM phosphate, 1 mM EDTA and 0.5% BSA for 10 min at room temperature. This was followed by two washes (10 min each wash) with the same buffer at 65°C, which was then followed by two washes (10 min each wash) at 65°C with 1% SDS, 25 mM phosphate and 1 mM EDTA. The blot was autoradiographed. The blot was reprobbed with a 100 nt cRNA probe to 18S rRNA as described above. Following autoradiography, the stromelysin expression was quantified on a scanning densitometer, which is followed by normalization of the data to the 18S rRNA band intensities.

As shown in Figs. 14-16, catalytically active 1049HH ribozyme mediates a decrease in the expression of stromelysin expression in rabbit knees. The inhibition appears to be sequence-specific and ranges from 50-70%.

Example 14: Phosphorothioate-substituted Ribozymes inhibit stromelysin expression in Rabbit Knee

Ribozymes containing four phosphorothioate linkages at the 5' termini enhance ribozyme efficacy in mammalian cells. Referring to Fig. 17, applicant has designed and synthesized hammerhead ribozymes targeted to site 1049 within stromelysin RNA, wherein, the ribozymes contain five phosphorothioate linkages at their 5' and 3' termini. Additionally, these ribozymes contain 2'-O-methyl substitutions at 30 nucleotide positions, 2'-C-allyl substitution at U4 position and 2'-OH at five positions (Fig 17A). As described above, these ribozymes were administered to rabbit knees to test for ribozyme efficacy. The 1049 U4-C-allyl P=S active ribozyme shows greater than 50 % reduction in the level of stromelysin RNA in rabbit knee. Catalytically inactive version of the 1049 U4-C-allyl P=S ribozyme shows ~30% reduction in the level of stromelysin RNA.

Referring to Fig. 18, applicant has also designed and synthesized hammerhead ribozymes targeted to three distinct sites within stromelysin RNA, wherein, the ribozymes contain four phosphorothioate linkages at their 5' termini. Additionally, these ribozymes contain 2'-O-methyl substitutions at 29  
5 nucleotide positions, 2'-amino substitutions at U4 and U7 positions and 2'-OH at five positions. As described above, these ribozymes were administered to rabbit knees to test for ribozyme efficacy. As shown in Figures 18-21, ribozymes targeted to sites 1049, 1363 and 1366 are all efficacious in rabbit  
10 knee. All three ribozymes decreased the level of stromelysin RNA in rabbit knee by about 50 %.

Sequences and chemical modifications described in figures 17 and 18 are meant to be non-limiting examples. Those skilled in the art will recognize that similar embodiments with other ribozymes and ribozymes containing other chemical modifications can be readily generated using techniques  
15 known in the art and are within the scope of the present invention.

Applicant has shown that chemical modifications, such as 6-methyl U and abasic (nucleotide containing no base) moieties can be substituted at certain positions within the ribozyme, for example U4 and U7 positions, without significantly effecting the catalytic activity of the ribozyme. Similarly,  
20 3'-3' linked abasic inverted ribose moieties can be used to protect the 3' ends of ribozymes in place of an inverted T without effecting the activity of the ribozyme.

B7-1, B7-2, B7-3 and CD40 are attractive ribozyme targets by several criteria. The molecular mechanism of T cell activation is well-established.  
25 Efficacy can be tested in well-defined and predictive animal models. The clinical end-point of graft rejection is clear. Since delivery would be *ex vivo*, treatment of the correct cell population would be assured. Finally, the disease condition is serious and current therapies are inadequate. Whereas protein-based therapies would induce anergy against all antigens encountered during  
30 the several week treatment period, *ex vivo* ribozyme therapy provides a direct and elegant approach to truly donor-specific anergy.



Similarly, autoimmune diseases and allergies can be prevented or treated by reversing the devastating course of immune response to self-antigens. Specifically, nucleic acids of this inventions can dampen the response to naturally occurring antigens.

5     Example 15: B7-1, B7-2, B7-3 and/or CD40 Hammerhead ribozymes

By engineering ribozyme motifs we have designed several ribozymes directed against B7-1, B7-2, B7-3 and/or CD40 encoded mRNA sequences. These ribozymes were synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave target sequences *in vitro* was evaluated.

Several common human cell lines are available that can be induced to express endogenous B7-1, B7-2, B7-3 and/or CD40. Alternatively, murine splenic cells can be isolated and induced, to express B7-1 or B7-2, with IL-4 or recombinant CD40 ligand. B7-1 and B7-2 can be detected easily with monoclonal antibodies. Use of appropriate fluorescent reagents and fluorescence-activated cell-sorting (FACS) will permit direct quantitation of surface B7-1 and B7-2 on a cell-by-cell basis. Active ribozymes are expected to directly reduce B7-1 or B7-2 expression. Ribozymes targeted to CD40 would prevent induction of B7-2 by CD40 ligand.

Several animal models of transplantation are available – Mouse, rat, Porcine model (Fodor et al., 1994, *Proc. Natl. Acad. Sci. USA* 91, 11153); or Baboon (reviewed by Nowak, 1994 *Science* 266, 1148). B7-1, B7-2, B7-3 and/or CD40 protein levels can be measured clinically or experimentally by FACS analysis. B7-1, B7-2, B7-3 and/or CD40 encoded mRNA levels will be assessed by Northern analysis, RNase-protection, primer extension analysis and/or quantitative RT-PCR. Ribozymes that block the induction of B7-1, B7-2, B7-3 and/or CD40 activity and/or B7-1, B7-2, B7-3 and/or CD40 protein encoding mRNAs by more than 20% *in vitro* will be identified.

Several animals models of autoimmune disorders are available– allergic encephalomyelitis (EAE) in Lewis rats (Carlson et al., 1993 *Ann. N.Y. Acad. Sci.* 685, 86); animal models of multiple sclerosis (Wekerle et al., 1994 *Ann.*

Neurol. 36, s47) and rheumatoid arthritis (van Laar et al., 1994 Chem. Immunol. 58, 206).

Several animal models of allergy are available and are reviewed by Kemeny and Diaz-Sanchez, 1990, Clin. Exp. Immunol. 82, 423 and Pretolani et al., 1994 Ann. N.Y.Acad. Sci. 725, 247).

RNA ribozymes and/or genes encoding them will be delivered by either free delivery, liposome delivery, cationic lipid delivery, adeno-associated virus vector delivery, adenovirus vector delivery, retrovirus vector delivery or plasmid vector delivery in these animal model experiments (see above). One dose of a ribozyme vector that constitutively expresses the ribozyme or one or more doses of a stable anti-B7-1, B7-2, B7-3 and/or CD40 ribozymes or a transiently expressing ribozyme vector to donor APC, followed by infusion into the recipient may reduce the incidence of graft rejection. Alternatively, graft tissues may be treated as described above prior to transplantation.

Example 16: Synthesis of 6-methyl-uridine phosphoramidite

Referring to Figure 30, the suspension of 6-methyl-uracil (2.77g, 21.96 mmol) in the mixture of hexamethyldisilazane (50mL) and dry pyridine (50mL) was refluxed for three hours. The resulting clear solution of trimethylsilyl derivative of 6-methyl uracyl was evaporated to dryness and coevaporated 2 times with dry toluene to remove traces of pyridine. To the solution of the resulting clear oil, in dry acetonitrile, 1-O-acetyl-2',3',5'-tri-O-benzoyl-b-D-ribose (10.1g, 20 mmol) was added and the reaction mixture was cooled to 0°C. To the above stirred solution, trimethylsilyl trifluoromethanesulfonate (4.35 mL, 24 mmol) was added dropwise and the reaction mixture was stirred for 1.5 h at 0°C and then 1h at room temperature. After that the reaction mixture was diluted with dichloromethane washed with saturated sodium bicarbonate and brine. The organic layer was evaporated and the residue was purified by flash chromatography on silica gel with ethylacetate-hexane (2:1) mixture as an eluent to give 9.5g (83%) of the compound 2 and 0.8g of the corresponding N<sup>1</sup>,N<sup>3</sup>-bis-derivative.

To the cooled (-10°C) solution of the compound (4.2g, 7.36 mmol) in the mixture of pyridine (60 mL) and methanol (10 mL) ice-cooled 2M aqueous

solution of sodium hydroxide (16 mL) was added with constant stirring. The reaction mixture was stirred at -10°C for additional 30 minutes and then neutralized to pH 7 with Dowex 50 (Py<sup>+</sup>). The resin was filtered off and washed with a 200 mL mixture of H<sub>2</sub>O - Pyridine (4:1). The combined "mother liquor" and the washings were evaporated to dryness and dried by multiple coevaporation with dry pyridine. The residue was redissolved in dry pyridine and then mixed with dimethoxytrityl chloride (2.99g, 8.03 mmol). The reaction mixture was left overnight at room temperature. Reaction was quenched with methanol (25 mL) and the mixture was evaporated. The residue was dissolved in dichloromethane, washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by flash chromatography on silica gel using linear gradient of MeOH (2% to 5%) in CH<sub>2</sub>Cl<sub>2</sub> as eluent to give 3.4g (83%) of the compound 6.

15 Example 17: Synthesis of 6-methyl-cytidine phosphoramidite

Triethylamine (13.4 ml, 100 mmol) was added dropwise to a stirred ice-cooled mixture of 1,2,4-triazole (6.22g, 90 mmol) and phosphorous oxychloride (1.89 ml, 20 mmol) in 50 ml of anhydrous acetonitrile. To the resulting suspension the solution of 2',3',5'-tri-O-Benzoyl-6-methyl uridine (5.7g, 10 mmol) in 30 ml of acetonitrile was added dropwise and the reaction mixture was stirred for 4 hours at room temperature. Then it was concentrated in vacuo to minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and the solvent was removed in vacuo. The residue was dissolved in 100 ml of 1,4-dioxane and treated with 50 mL of 29% aq NH<sub>4</sub>OH overnight. The solvents were removed in vacuo. The residue was dissolved in the mixture of pyridine (60 mL) and methanol (10 mL), cooled to -15°C and ice-cooled 2M aq solution of sodium hydroxide was added under stirring. The reaction mixture was stirred at -10 to -15°C for additional 30 minutes and then neutralized to pH 7 with Dowex 50 (Py<sup>+</sup>). The resin was filtered off and washed with 200 mL of the mixture H<sub>2</sub>O - Py (4:1). The combined mother liquor and washings were evaporated to dryness. The residue was crystallized from aq methanol to give 1.6g (62%) of 6-methyl cytidine.

To the solution of 6-methyl cytidine (1.4g, 5.44 mmol) in dry pyridine 3.11 mL of trimethylchlorosilane was added and the reaction mixture was stirred for 2 hours at room temperature. Then acetic anhydride (0.51 mL, 5.44 mmol) was added and the reaction mixture was stirred for additional 3 hours at room temperature. TLC showed disappearance of the starting material and the reaction was quenched with MeOH (20 mL), ice-cooled and treated with water (20 mL, 1 hour). The solvents were removed in vacuo and the residue was dried by four coevaporations with dry pyridine. Finally it was redissolved in dry pyridine and dimethoxytrityl chloride (2.2 g, 6.52 mmol) was added. The reaction mixture was stirred overnight at room temperature and quenched with MeOH (20 mL). The solvents were removed in vacuo. The remaining oil was dissolved in methylene chloride, washed with saturated sodium bicarbonate and brine. The organic layer was separated and evaporated and the residue was purified by flash chromatography on silica gel with the gradient of MeOH in methylene chloride (3% to 5%) to give 2.4 g (74%) of the compound (4).

Example 18: Synthesis of 6-aza-uridine and 6-aza-cytidine

To the solution of 6-aza uridine (5g, 20.39 mmol) in dry pyridine dimethoxytrityl chloride (8.29g, 24.47 mmol) was added and the reaction mixture was left overnight at room temperature. Then it was quenched with methanol (50 mL) and the solvents were removed in vacuo. The remaining oil was dissolved in methylene chloride and washed with saturated aq sodium bicarbonate and brine. The organic layer was separated and evaporated to dryness. The residue was additionally dried by multiple coevaporations with dry pyridine and finally dissolved in dry pyridine. Acetic anhydride (4.43 mL, 46.7 mmol) was added to the above solution and the reaction mixture was left for 3 hours at room temperature. Then it was quenched with methanol and worked-up as above. The residue was purified by flash chromatography on silica gel using mixture of 2% of MeOH in methylene chloride as an eluent to give 9.6g (75%) of the compound.

Triethylamine (23.7 mL, 170.4 mmol) was added dropwise to a stirred ice-cooled mixture of 1,2,4-triazole (10.6g, 153.36 mmol) and phosphorous oxychloride (3.22 mL, 34.08 mmol) in 100 mL of anhydrous acetonitrile. To the resulting suspension the solution of 2',3'-di-O-Acetyl-5'-O-Dimethoxytrityl-6-

aza Uridine (7.13g, 11.36 mmol) in 40 ml of acetonitril was added dropwise and the reaction mixture was stirred for 6 hours at room temperature. Then it was concentrated in vacuo to minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and the solvent was removed in vacuo. The residue was dissolved in 150 ml of 1,4-dioxane and treated with 50 mL of 29% aq  $\text{NH}_4\text{OH}$  for 20 hours at room temperature. The solvents were removed in vacuo. The residue was purified by flash chromatography on silica gel using linear gradient of MeOH (4% to 10%) in methylene chloride as an eluent to give 3.1g (50%) of azacytidine.

To the stirred solution of 5'-O-Dimethoxytrityl-6-aza cytidine (3g, 5.53 mmol) in anhydrous pyridine trimethylchloro silane (2.41 mL, 19 mmol) was added and the reaction mixture was left for 4 hours at room temperature. Then acetic anhydride (0.63 mL, 6.64 mmol) was added and the reaction mixture was stirred for additional 3 hours at room temperature. After that it was quenched with MeOH (15 mL) and the solvents were removed in vacuo. The residue was treated with 1M solution of tetrabutylammonium fluoride in THF (20°, 30 min) and evaporated to dryness.. The remaining oil was dissolved in methylene chloride, washed with saturated aq sodium bicarbonate and water. The separated organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by flash chromatography on silica gel using 4% MeOH in methylene chloride as an eluent to give 2.9g (89.8%) of the compound.

General Procedure for the Introducing of the TBDMS-Group: To the stirred solution of the protected nucleoside in 50 mL of dry THF and pyridine (4 eq)  $\text{AgNO}_3$  (2.4 eq) was added. After 10 minutes tert-butyldimethylsilyl chloride (1.5 eq) was added and the reaction mixture was stirred at room temperature for 12 hours. The resulted suspension was filtered into 100 mL of 5% aq  $\text{NaHCO}_3$ . The solution was extracted with dichloromethane (2x100 mL). The combined organic layer was washed with brine, dried over  $\text{Na}_2\text{SO}_4$  and evaporated. The residue was purified by flash chromatography on silica gel with hexanes-ethylacetate (3:2) mixture as eluent.

General Procedure for Phosphitylation: To the ice-cooled stirred solution of protected nucleoside (1 mmol) in dry dichloromethane (20 mL) under argon blanket was added dropwise via syringe the premixed solution of N,N-diisopropylethylamine (2.5eq) and 2-cyanoethyl N'-N-diisopropylchlorophosphoramidite (1.2 eq) in dichloromethane (3 mL).  
5 Simultaneously via another syringe N-methylimidazole (1 eq) was added and stirring was continued for 2 hours at room temperature. After that the reaction mixture was again ice-cooled and quenched with 15 ml of dry methanol. After 5 min stirring, the mixture was concentrated in vacuo (<40°C) and purified by  
10 flash chromatography on silica gel using hexanes-ethylacetate mixture contained 1% triethylamine as an eluent to give corresponding phosphoroamidite as white foam.

Example 19: RNA cleavage activity of HHA ribozyme substituted with 6-methyl-Uridine

15 Hammerhead ribozymes targeted to site A (see Fig. 31) were synthesized using solid-phase synthesis, as described above. U4 position was modified with 6-methyl-uridine.

RNA cleavage assay *in vitro*:

Substrate RNA is 5' end-labeled using [ $\gamma$ -<sup>32</sup>P] ATP and T4 polynucleotide  
20 kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace amount ( $\leq 1$  nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme are denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10 -15 min. The ribozyme and substrate are  
25 incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl and 10 mM MgCl<sub>2</sub>. The reaction is initiated by mixing the ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5  $\mu$ l are taken at regular intervals of time and the reaction is quenched by mixing with equal volume of 2X formamide stop mix. The samples are resolved on 20 % denaturing polyacrylamide gels.  
30 The results are quantified and percentage of target RNA cleaved is plotted as a function of time.

Referring to Fig. 32, hammerhead ribozymes containing 6-methyl-uridine modification at U4 position cleave the target RNA efficiently.

Example 20: RNA cleavage activity of HHB ribozyme substituted with 6-methyl-Uridine

Hammerhead ribozymes targeted to site B (see Fig. 33) were synthesized using solid-phase synthesis, as described above. U4 and U7 positions were  
5 modified with 6-methyl-uridine.

RNA cleavage reactions were carried out as described above. Referring to Fig. 34, hammerhead ribozymes containing 6-methyl-uridine-modification at U4 and U7 positions cleave the target RNA efficiently.

Example 21: RNA cleavage activity of HHC ribozyme substituted with 6-methyl-Uridine

Hammerhead ribozymes targeted to site C (see Fig. 35) were synthesized using solid-phase synthesis, as described above. U4 and U7 positions were  
10 modified with 6-methyl-uridine.

RNA cleavage reactions were carried out as described above. Referring to  
15 Fig. 36, hammerhead ribozymes containing 6-methyl-uridine modification at U4 positions cleave the target RNA efficiently.

Sequences listed in Figure 23, 31, 33, 35, and others and the modifications described in these figures are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions, mutations, chemical modifications) of the ribozyme and RNA containing other 2'-  
20 hydroxyl group modifications, including but not limited to amino acids, peptides and cholesterol, can be readily generated using techniques known in the art, and are within the scope of the present invention.

Example 22: Inhibition of Rat smooth muscle cell proliferation by 6-methyl-U substituted ribozyme HHA.

Hammerhead ribozyme (HHA) is targeted to a unique site (site A) within c-*myb* mRNA. Expression of c-*myb* protein has been shown to be essential for the proliferation of rat smooth muscle cell (Brown *et al.*, 1992 *J. Biol. Chem.* 267, 4625).  
25

The ribozymes that cleaved site A within *c-myc* RNA described above were assayed for their effect on smooth muscle cell proliferation. Rat vascular smooth muscle cells were isolated and cultured as described (Stinchcomb *et al.*, *supra*). HHA ribozymes were complexed with lipids and delivered into rat smooth muscle cells. Serum-starved cells were stimulated as described by Stinchcomb *et al.*, *supra*. Briefly, serum-starved smooth muscle cells were washed twice with PBS, and the RNA/lipid complex was added. The plates were incubated for 4 hours at 37°C. The medium was then removed and DMEM containing 10% FBS, additives and 10 µM bromodeoxyuridine (BrdU) was added. In some wells, FBS was omitted to determine the baseline of unstimulated proliferation. The plates were incubated at 37°C for 20-24 hours, fixed with 0.3% H<sub>2</sub>O<sub>2</sub> in 100% methanol, and stained for BrdU incorporation by standard methods. In this procedure, cells that have proliferated and incorporated BrdU stain brown; non-proliferating cells are counter-stained a light purple. Both BrdU positive and BrdU negative cells were counted under the microscope. 300-600 total cells per well were counted. In the following experiments, the percentage of the total cells that have incorporated BrdU (% cell proliferation) is presented. Errors represent the range of duplicate wells. Percent inhibition then is calculated from the % cell proliferation values as follows: % inhibition = 100 - 100 (Ribozyme - 0% serum)/(Control - 0% serum).

Referring to Figure 37, active ribozymes substituted with 6-methyl-U at position 4 of HHA were successful in inhibiting rat smooth muscle cell proliferation. A catalytically inactive ribozyme (inactive HHA), which has two base substitutions within the core (these mutations inactivate a hammerhead ribozyme; Stinchcomb *et al.*, *supra*), does not significantly inhibit rat smooth muscle cell proliferation.

Example 23: Inhibition of stromelysin production in human synovial fibroblast cells by 6-methyl-U substituted ribozyme HHC.

Hammerhead ribozyme (HHC) is targeted to a unique site (site C) within *stromelysin* mRNA.

The general assay was as described (Draper *et al.*, *supra*). Briefly, fibroblasts, which produce stromelysin, are serum-starved overnight and ribozymes or controls are offered to the cells the next day. Cells were maintained in serum-free media. The ribozyme were applied to the cells as free ribozyme, or in association with various delivery vehicles such as cationic



lipids (including Transfectam™, Lipofectin™ and Lipofectamine™), conventional liposomes, non-phospholipid liposomes or biodegradable polymers. At the time of ribozyme addition, or up to 3 hours later, Interleukin-1 $\alpha$  (typically 20 units/ml) can be added to the cells to induce a large increase in stromelysin expression. The production of stromelysin can then be monitored over a time course, usually up to 24 hours.

Supernatants were harvested 16 hours after IL-1 induction and assayed for stromelysin expression by ELISA. Polyclonal antibody against Matrix Metalloproteinase 3 (Biogenesis, NH) was used as the detecting antibody and anti-stromelysin monoclonal antibody was used as the capturing antibody in the sandwich ELISA (Maniatis *et al.*, *supra*) to measure stromelysin expression.

Referring to Figure 38, HHC ribozyme containing 6-methyl-U modification, caused a significant reduction in the level of stromelysin protein production. Catalytically inactive HHC had no significant effect on the protein level.

Example 24: Synthesis of pyridin-2(4)-one nucleoside 3'-phosphoramidites

General procedure for the preparation of 1-(2,3,5-tri-O-benzoyl- $\beta$ -D-ribofuranosyl)-2(4)-pyridones (3) and (9)

Referring to Figure 39, 2- or 4-hydroxypyridine (1) or (8) (2.09 g, 22 mmol), 1-O-acetyl-2,3,5-tri-O-benzoyl- $\beta$ -D-ribofuranose (2) (10.08 g, 20 mmol) and BSA (5.5 ml, 22 mmol) were dissolved in dry acetonitrile (100 ml) under argon at 70°C (oil bath) and the mixture stirred for 10 min. Trimethylsilyl trifluoromethanesulfonate (TMSTf) (5.5 ml, 28.5 mmol) was added and the mixture was stirred for an additional hour for 1 or four hours for 8. The mixture was then cooled to room temperature (RT) followed by dilution, with CHCl<sub>3</sub> (200 ml), and extraction, with sat. aq. NaHCO<sub>3</sub> solution. The organic layer was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to dryness *in vacuo*. The residue was chromatographed on the column of silica gel; 1-5% gradient of methanol in dichloromethane was used for purification of 3 (98% yield) and 2-10% gradient of methanol in dichloromethane for purification of 9 (84% yield).

1-( $\beta$ -D-Ribofuranosyl)-2(4)-pyridones (4) and (10)

3 or 9 (18 mmol) was dissolved in 0.3M NaOCH<sub>3</sub> (150 ml) and the solution was stirred at RT for 1 hour. The mixture was then neutralized, with Dowex 50WX8 (Py<sup>+</sup>), the ion-exchanger was filtered off and the filtrate was concentrated to a syrup *in vacuo*. The residue was dissolved in water (100 ml) and the solution was washed with chloroform (2 x 50 ml) and ether (2 x 50 ml). The aqueous layer was evaporated to dryness and the residue was then crystallized from ethyl acetate (3.9 g, 91% 4; Niedballa *et al.*, *Nucleic Acid Chemistry*, Part 1, Townsend, L.B. and Tipson, R.S., Ed.; J. Wiley & Sons, Inc.; New York, 1978, p 481-484); 10 (Niedballa and Vorbrüggen, *J. Org. Chem.* 1974, 39, 3668-3671) was crystallized from ethanol (3.6 g, 84%).

1-(2-O-TBDMSi-5-O-DMT- $\beta$ -D-ribofuranosyl)-2(4)-pyridones

4 or 10 was 5'-O-dimethoxytritylated according to the standard procedure (see Oligonucleotide Synthesis: A Practical Approach, M.J. Gait Ed.; IRL Press, Oxford, 1984, p 27) to yield 5 in 76% yield and pyridin-4-one derivative in 67% yield in the form of yellowish foams after silica gel column chromatography (0.5-10% gradient of methanol in dichloromethane). These compounds were treated with *t*-butyldimethylsilyl chloride under the conditions described by Hakimelahi *et al.*, *Can. J. Chem.* 1982, 60, 1106-1113, and the reaction mixtures were purified by the silica gel column chromatography (20-50% gradient of ethyl acetate in hexanes) to enable faster moving 2'-O-TBDMSi isomers (68.5% and 55%, respectively) as colorless foams.

1-[2-O-*t*-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)]-2(4)-pyridones (7) and (11)

1-(2-O-TBDMS-5-O-DMT- $\beta$ -D-ribofuranosyl)-2(4)-pyridones were phosphitylated under conditions described by Tuschl *et al.*, *Biochemistry* 1993, 32, 11658-11668, and the products were isolated by silica gel column chromatography using 15-50% gradient of ethyl acetate in hexanes (1% Et<sub>3</sub>N) for 7 (89% yield) and dichloromethane (1% Et<sub>3</sub>N) for 11 (94% yield).

Phosphoramidites 7 and 11 were incorporated into ribozymes and substrates using the method of synthesis, deprotection, purification and testing

was purified by silica gel column chromatography (2-10% gradient of ethyl acetate in hexanes) to afford a slower moving 2'-O-TBDMSi isomer 7 (0.6 g, 35% yield) as a colorless foam. The faster migrating 3'-O-TBDMSi isomer 6 was also isolated (0.55 g, 32% yield).

5 2-O-*t*-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)-1-deoxy-1-phenyl- $\beta$ -D-ribofuranose (8)

Compound 7 (0.87 g, 1.39 mmol) was phosphitylated under conditions described by Tuschl *et al.*, *supra* and the product was isolated by silica gel column chromatography using 0.5% ethyl acetate in toluene (1% Et<sub>3</sub>N) for elution  
10 (0.85 g, 74% yield).

Example 26: Synthesis of pseudouridine, 3-methyluridine and 2,4,6-trimethoxy benzene nucleoside phosphoramidites

Starting with a pseudo uridine, 3-methyluridine or 2,4,6-trimethoxy benzene nucleoside (Gasparutto *et al.*, *Nucleic Acid Res.* 1992 20, 5159-5166; Kalvoda  
15 and Farkas, *Nucleic Acid Chemistry*, Part 1, Townsend, L.B. and Tipson, R.S., Ed.: J. Wiley & Sons, Inc.; New York, 1978, p 481-484), phosphoramidites can be prepared by standard protocols described below (Figure 41).

General Procedure for the Introducing of the TBDMS-Group: To the stirred solution of the protected nucleoside in 50 mL of dry THF and pyridine (4 eq)  
20 AgNO<sub>3</sub> (2.4 eq) was added. After 10 minutes tert-butyldimethylsilyl chloride (1.5 eq) was added and the reaction mixture was stirred at room temperature for 12 hours. The resulted suspension was filtered into 100 mL of 5% aq NaHCO<sub>3</sub>. The solution was extracted with dichloromethane (2x100 mL). The combined organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. The residue  
25 was purified by flash chromatography on silica gel with hexanes-ethylacetate (3:2) mixture as eluent.

General Procedure for Phosphitylation: To the ice-cooled stirred solution of protected nucleoside (1 mmol) in dry dichloromethane (20 mL) under argon blanket was added dropwise via syringe the premixed solution of N,N-diisopropylethylamine (2.5eq) and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (1.2 eq) in dichloromethane (3 mL).  
30

Simultaneously via another syringe N-methylimidazole (1 eq) was added and stirring was continued for 2 hours at room temperature. After that the reaction mixture was again ice-cooled and quenched with 15 ml of dry methanol. After 5 min stirring, the mixture was concentrated in vacuo (<40°C) and purified by flash chromatography on silica gel using hexanes-ethylacetate mixture contained 1% triethylamine as an eluent to give corresponding phosphoroamidite as white foam.

Pseudouridine, 3-methyluridine or 2,4,6-trimethoxy benzene phosphoramidites were incorporated into ribozymes using solid phase synthesis as described by Wincott *et al.*, 1995 *supra*. The ribozymes were deprotected using the standard protocol described above with the exception of ribozymes with pseudouridine. Pseudouridine-modified ribozymes were deprotected first by incubation at room temperature, instead of at 55°C, for 24 hours in a mixture of ethanolic ammonia (3:1).

Example 27: Synthesis of dihydrouridine phosphoramidites

Referring to Figure 42, dihydrouridine phosphoramidite was synthesized based on the method described in Chaix *et al.*, 1989 *Nucleic Acid Res.* 17, 7381-7393 with certain improvements:

i. Uridine (1; 10g, 41mmoles) was dissolved in 200 ml distilled water and to the solution 2g of Rh (10% on alumina) was added. The slurry was brought to 60 psi of hydrogen, and hydrogenation was continued for 16hrs. Reaction was monitored by disappearance of UV absorbing material. All of starting material was converted to dihydrouridine (DHU) and tetrahydrouridine (2:1 based on NMR). Tetrahydrouridine was not removed at this step.

ii. Dihydrouridine (2; 10g, 41mmoles) was dissolved in 400ml dry pyridine; dimethylaminopyridine (0.244g, 2mmoles), triethylamine (7.93ml, 56mmoles), and dimethoxytritylchloride (16.3g, 48mmoles) were added and stirred under argon overnight. The reaction was quenched with 50ml methanol, extracted with 400ml 5% sodium bicarbonate, and then 400ml brine. The organic phase was dried over sodium sulphate, filtered, and then dried to a foam. 5'-DMT-DHU (3) was purified by silica gel chromatography (dichloromethane with 0.5-5% gradient of methanol; final yield = 9g; 16.4mmoles).

III. 5'-DMT-DHU (3; 9.0g, 16.4mmoles) was dissolved in 150ml dry THF. Pyridine (4.9ml, 60mmoles) and silver nitrate (3.35g, 19.7mmoles) were added at room temperature and stirred under argon for 10min., then tert.-butyldimethylsilylchloride (tBDMS-Cl; 3.0g, 19.7mmoles) was added and the slurry was stirred under argon overnight. The reaction was filtered over celite into 500ml aqueous 5% sodium bicarbonate and then extracted with 200ml chloroform. The organic phase was washed with 250ml brine, dried over sodium sulfate, and then evaporated to a yellow foam. 2'-tBDMS, 5'-DMT-DHU (5) was purified by silica gel chromatography away from the 3'-tBDMS, 5'-DMT-DHU (4) (hexanes with 10-50% gradient ether; final yield = 5.1g; 7.7mmoles), dried over sodium sulfate, filtered, and then dried to a white powder. The product was kept under high vacuum for 48hrs.

IV. 5'-DMT, 2'-tBDMS-DHU (5; 2.10g, 3.17mmoles) was dissolved in 40ml anhydrous dichloromethane. NN-dimethylaminopyridine (2.21ml, 12.7mmoles), N-methylimidazole (1.27ml, 1.59mmoles), and chloro-diisopropylcyanoethylphosphoramidite (1.2ml, 5.22mmoles) were added and the reaction was stirred under argon for 3hrs. The reaction was quenched with 4ml anhydrous methanol and then evaporated to an oil. Final product (6) was purified by silica gel chromatography (dichloromethane with 0-1% ethanol; 1% triethylamine; final yield = 2.2g; 2.5mmoles).

The dihydrouridine was incorporated into ribozymes using solid phase synthesis as described by Wincott *et al.*, 1995 *supra.* with improvements—nucleoside-oxalyl-polystyrene derivatized support (Alul *et. al.* Nucleic Acids Res., 1991, 19, 1527-1532) was used. The ribozyme containing the dihydrouridine substitution was deprotected using 30% methyl amine in anhydrous ethanol for 15 min. at room temperature and subsequent treatment with *tert*-butyl-ammonium fluoride in anhydrous THF for 24 hrs. at room temperature.

Example 28: Synthesis of 2-O-t-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)-1-deoxy-1-naphthyl-β-D-ribofuranose (7) phosphoramidites

1-Deoxy-1-naphthyl-β-D-ribofuranose (4)

Referring to Figure 45, the title compound was synthesized from naphthalen 1 and tetra-*O*-acetyl- $\beta$ -D-ribofuranose 2 according to the procedure of Ohruj *et al.* *Agr. Biol. Chem.* 1972, 36, 1651-1653.

5 2-*O*-*t*-Butyldimethylsilyl-5-*O*-dimethoxytrityl-3-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-deoxy-1-naphthyl- $\beta$ -D-ribofuranose (7)

7 was synthesized in three steps from 4: a) 5'-*O*-dimethoxytritylation using 4,4'-dimethoxytrityl triflate, followed by chromatographic separation of  $\alpha$  and  $\beta$  anomer, respectively; b) 2'-*O*-silylation was carried out as described by Hakimelahi *et al.*, 1982 *supra* (32% yield); c) 3'-*O*-phosphitylation was carried  
10 out essentially as described by Tuschl *et al.*, 1993 *supra* (85% yield).

This phosphoramidite is incorporated into ribozymes using solid phase synthesis as described by Wincott *et al.*, 1995 *supra*. The ribozyme containing naphthyl substitution was deprotected using the standard protocol described above.

15 Example 29: Synthesis of 2-*O*-*t*-Butyldimethylsilyl-5-*O*-Dimethoxytrityl-3-*O*-(2-Cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-Deoxy-1-(*p*-Aminophenyl)- $\beta$ -D-Ribofuranose phosphoramidites

5-*O*-*t*-Butyldiphenylsilyl-2,3-*O*-isopropylidene-1-deoxy-1-(*p*-bromophenyl)- $\beta$ -D-ribofuranose (3)

20 Referring to Figure 46, 3 was prepared from 4-bromo-1-lithiobenzene and *t*-butyldiphenylsilyl-2,3-*O*-isopropylidene-D-ribo-1,4-lactone using the procedure analogous to that described by Czerniecki and Ville, *J. Org. Chem.* 1989, 54, 610-612. Contrary to their result, we succeeded in obtaining the title compound, by using instead of *t*-butyldimethylsilyl the more acid resistant  
25 *t*-butyldiphenylsilyl group for 5-*O*-protection.

5-*O*-*t*-Butyldiphenylsilyl-2,3-*O*-isopropylidene-1-deoxy-1-(*p*-aminophenyl)- $\beta$ -D-ribofuranose (5)

Compound 3 was aminated using liquid ammonia and CuI as described by Piccirilli *et al.* *Helv. Chim. Acta* 1991, 74, 397-406 to give the title  
30 compound in 63% yield.

5-O-t-Butyldiphenylsilyl-2,3-O-isopropylidene-1-deoxy-1-[p-(N-TFA) aminophenyl]- $\beta$ -D-ribofuranose (6)

5 (1.2 g, 2.88 mmol) in dry pyridine (20 ml) was treated with trifluoroacetic anhydride (0.5 ml, 3.6 mmol) for 1 hour at 0 °C. The reaction mixture was then quenched with methanol (5 ml) and evaporated to a syrup. The syrup was partitioned between 5% aq. NaHCO<sub>3</sub> and dichloromethane, organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to dryness under reduced pressure. This material was used without further purification in the next step.

1-Deoxy-1-[p-(N-TFA)aminophenyl]- $\beta$ -D-ribofuranose (7)

10 The title compound was prepared from 6 in an identical manner as for the synthesis of deblocked phenyl analog; (82% overall yield for 5'-O-desilylation and the cleavage of 2',3'-O-isopropylidene group).

2-O-t-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)-1-deoxy-1-[p-(N-TFA) aminophenyl]- $\beta$ -D-ribofuranose (10)

15 Using the same three step sequence as for the phenyl analog, 10 was prepared from 7 in 32% overall yield.

20 This phosphoramidite is incorporated into ribozymes using solid phase synthesis as described by Wincott *et al.*, 1995 *supra*. The ribozyme containing aminophenyl substitution was deprotected using the standard protocol described above.

Example 30: RNA cleavage reactions catalyzed by HH-B substituted with modified bases

25 Hammerhead ribozymes targeted to site B (see Fig. 43A) were synthesized using solid-phase synthesis, as described above. U4 and U7 positions were substituted with various base-modifications shown in Figure 43B.

30 RNA cleavage reactions were carried out as described above. Referring to Fig. 43B, hammerhead ribozymes containing base modifications at positions 4 or 7 cleave the target RNA to varying degrees of efficiency. Some of the base modifications at position 7 appear to enhance the catalytic efficiency of the

hammerhead ribozymes compared to a standard base at that position (see Figure 43B, pyridin-4-one, phenyl and 3-methyl U modifications).

5 HH-B ribozymes with either pyridin-4-one or phenyl substitution at position 7 were further characterized (Figure 44). It appears that HH-B ribozyme with pyridin-4-one modification at position 7 cleaves RNA with a 10 fold higher  $k_{cat}$  when compared to a ribozyme with a U at position 7 (compare Figure 44 A with 44 B). HH-B ribozyme with a phenyl group at position 7 cleaves RNA with a 3 fold higher  $k_{cat}$  when compared to a hammerhead ribozyme with U at position 7 (see Figure 44C).

10 Sequences listed in Figure 23, 31, 33, 35, 43 and the modifications described in these figures are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions, mutations, chemical modifications) of the ribozyme and RNA containing other 2'-hydroxyl group modifications, including but not limited to amino acids, peptides  
15 and cholesterol, can be readily generated using techniques known in the art, and are within the scope of the present invention.

Example 31: 2'-deoxy-2'-alkylnucleotides

Table D2 is a summary of specified catalytic parameters ( $t_A$  and  $t_S$ ) on short substrates *in vitro*, and stabilities of the noted modified catalytic nucleic  
20 acids in human serum. U4 and U7 refer to the uracil bases noted in Figure 1. Modifications at the 2'-position are shown in the table.



Table D2

Entry	Modification	$t_{1/2}$ (m) Activity ( $t_A$ )	$t_{1/2}$ (m) Stability ( $t_S$ )	$\beta = t_S/t_A$ $\times 10$
1	U4 & U7 = U	1	0.1	1
2	U4 & U7 = 2'-O-Me-U	4	260	650
3	U4 = 2'=CH <sub>2</sub> -U	6.5	120	180
4	U7 = 2'=CH <sub>2</sub> -U	8	280	350
5	U4 & U7 = 2'=CH <sub>2</sub> -U	9.5	120	130
6	U4 = 2'=CF <sub>2</sub> -U	5	320	640
7	U7 = 2'=CF <sub>2</sub> -U	4	220	550
8	U4 & U7 = 2'=CF <sub>2</sub> -U	20	320	160
9	U4 = 2'-F-U	4	320	800
10	U7 = 2'-F-U	8	400	500
11	U4 & U7 = 2'-F-U	4	300	750
12	U4 = 2'-C-Allyl-U	3	>500	>1700
13	U7 = 2'-C-Allyl-U	3	220	730
14	U4 & U7 = 2'-C-Allyl-U	3	120	400
15	U4 = 2'-araF-U	5	>500	>1000
16	U7 = 2'-araF-U	4	350	875
17	U4 & U7 = 2'-araF-U	15	500	330
18	U4 = 2'-NH <sub>2</sub> -U	10	500	500
19	U7 = 2'-NH <sub>2</sub> -U	5	500	1000
20	U4 & U7 = 2'-NH <sub>2</sub> -U	2	300	1500
21	U4 = dU	6	100	170
22	U4 & U7 = dU	4	240	600

Figure 47 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided. Referring to Figure 47, the preferred sequence of a hammerhead ribozyme in a 5'- to 3'-direction of the catalytic core is CUGANGAG[base paired with]CGAAA. In this invention, the use of 2'-C-alkyl substituted nucleotides that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead ribozyme is described. Although substitutions of any nucleotide with any of the modified nucleotides shown in Figure 48 are possible, and were indeed synthesized, the basic structure composed of primarily 2'-O-Me nucleotides with selected substitutions was chosen to maintain maximal catalytic activity (Yang *et al. Biochemistry* 1992, 31, 5005-5009 and Paoletta *et al. EMBO J.* 1992, 11, 1913-1919) and ease of synthesis, but is not limiting to this invention.

Ribozymes from Figure 47 and Table D2 were synthesized and assayed for catalytic activity and nuclease resistance. With the exception of entries 8 and 17, all of the modified ribozymes retained at least 1/10 of the wild-type catalytic activity. From Table D2, all 2'-modified ribozymes showed very large and significant increases in stability in human serum (shown) and in the other fluids described below (Example 3, data not shown). The order of most aggressive nuclease activity was fetal bovine serum > human serum > human plasma > human synovial fluid. As an overall measure of the effect of these 2'-substitutions on stability and activity, a ratio  $\beta$  was calculated (Table D2). This  $\beta$  value indicated that all modified ribozymes tested had significant, >100 - >1700 fold, increases in overall stability and activity. These increases in  $\beta$  indicate that the lifetime of these modified ribozymes *in vivo* are significantly increased which should lead to a more pronounced biological effect.

More general substitutions of the 2'-modified nucleotides from Figure 48 also increased the  $t_{1/2}$  of the resulting modified ribozymes. However the catalytic activity of these ribozymes was decreased > 10-fold.

In Figure 53 compound 37 may be used as a general intermediate to prepare derivatized 2'-C-alkyl phosphoramidites, where X is CH<sub>3</sub>, or an alkyl, or other group described above.

The following are other non-limiting examples showing the synthesis of nucleic acids using 2'-C-alkyl substituted phosphoramidites, the syntheses of the amidites, their testing for enzymatic activity and nuclease resistance. These examples are diagrammed in Figs 48-54.

5    Example 32: Synthesis of Hammerhead Ribozymes Containing 2'-Deoxy-2'-Alkynucleotides & Other 2'-Modified Nucleotides

The method of synthesis used generally follows the procedure for normal RNA synthesis as described in Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* **1987**, *109*, 7845-7854 and in  
10    Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucleic Acids Res.* **1990**, *18*, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end (compounds 10, 12, 17, 22, 31, 18, 26, 32, 36 and 38). Other 2'-modified phosphoramidites were prepared according to: 3 & 4, Eckstein *et al.*  
15    *International Publication No. WO 92/07065*; and 5 Kois *et al. Nucleosides & Nucleotides* **1993**, *12*, 1093-1109. The average stepwise coupling yields were ~98%. The 2'-substituted phosphoramidites were incorporated into hammerhead ribozymes as shown in Figure 5. However, these 2'-alkyl substituted phosphoramidites may be incorporated not only into hammerhead  
20    ribozymes, but also into hairpin, hepatitis delta virus, Group I or Group II intron catalytic nucleic acids, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 33: Ribozyme Activity Assay

Purified 5'-end labeled RNA substrates (15-25-mers) and purified 5'-end  
25    labeled ribozymes (~36-mers) were both heated to 95 °C, quenched on ice and equilibrated at 37 °C, separately. Ribozyme stock solutions were 1 mM, 200 nM, 40 nM or 8 nM and the final substrate RNA concentrations were ~ 1 nM. Total reaction volumes were 50 mL. The assay buffer was 50 mM Tris-Cl, pH 7.5 and 10 mM MgCl<sub>2</sub>. Reactions were initiated by mixing substrate and  
30    ribozyme solutions at t = 0. Aliquots of 5 mL were removed at time points of 1, 5, 15, 30, 60 and 120 m. Each time point was quenched in formamide loading buffer and loaded onto a 15% denaturing polyacrylamide gel for analysis.

Quantitative analyses were performed using a phosphorimager (Molecular Dynamics).

Example 34: Stability Assay

500 pmol of gel-purified 5'-end-labeled ribozymes were precipitated in ethanol and pelleted by centrifugation. Each pellet was resuspended in 20 mL of appropriate fluid (human serum, human plasma, human synovial fluid or fetal bovine serum) by vortexing for 20 s at room temperature. The samples were placed into a 37 °C incubator and 2 mL aliquots were withdrawn after incubation for 0, 15, 30, 45, 60, 120, 240 and 480 m. Aliquots were added to 20 mL of a solution containing 95% formamide and 0.5X TBE (50 mM Tris, 50 mM borate, 1 mM EDTA) to quench further nuclease activity and the samples were frozen until loading onto gels. Ribozymes were size-fractionated by electrophoresis in 20% acrylamide/8M urea gels. The amount of intact ribozyme at each time point was quantified by scanning the bands with a phosphorimager (Molecular Dynamics) and the half-life of each ribozyme in the fluids was determined by plotting the percent intact ribozyme vs the time of incubation and extrapolation from the graph.

Example 35: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-O-Phenoxythio-carbonyl-Uridine (7)

To a stirred solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-uridine, 6, (15.1 g, 31 mmol, synthesized according to *Nucleic Acid Chemistry*, ed. Leroy Townsend, 1986 pp. 229-231) and dimethylaminopyridine (7.57 g, 62 mmol) a solution of phenylchlorothionoformate (5.15 mL, 37.2 mmol) in 50 mL of acetonitrile was added dropwise and the reaction stirred for 8 h. TLC (EtOAc:hexanes / 1:1) showed disappearance of the starting material. The reaction mixture was evaporated, the residue dissolved in chloroform, washed with water and brine, the organic layer was dried over sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography on silica gel with EtOAc:hexanes / 2:1 as eluent to give 16.44 g (85%) of 7.

Example 36: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-C-Allyl -Uridine (8)

To a refluxing, under argon, solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-2'-O-phenoxythiocarbonyl-uridine, **7**, (5 g, 8.03 mmol) and allyltributyltin (12.3 mL, 40.15 mmol) in dry toluene, benzoyl peroxide (0.5 g)  
5 was added portionwise during 1 h. The resulting mixture was allowed to reflux under argon for an additional 7-8 h. The reaction was then evaporated and the product **8** purified by flash chromatography on silica gel with EtOAc:hexanes / 1:3 as eluent. Yield 2.82 g (68.7%).

Example 37: 5'-O-Dimethoxytrityl-2'-C-Allyl-Uridine (9)

10 A solution of **8** (1.25 g, 2.45 mmol) in 10 mL of dry tetrahydrofuran (THF) was treated with a 1 M solution of tetrabutylammoniumfluoride in THF (3.7 mL) for 10 m at room temperature. The resulting mixture was evaporated, the residue was loaded onto a silica gel column, washed with 1 L of chloroform, and the desired deprotected compound was eluted with chloroform:methanol /  
15 9:1. Appropriate fractions were combined, solvents removed by evaporation, and the residue was dried by coevaporation with dry pyridine. The oily residue was redissolved in dry pyridine, dimethoxytritylchloride (1.2 eq) was added and the reaction mixture was left under anhydrous conditions overnight. The reaction was quenched with methanol (20 mL), evaporated,  
20 dissolved in chloroform, washed with 5% aq. sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by flash chromatography on silica gel, EtOAc:hexanes / 1:1 as eluent, to give 0.85 g (57%) of **9** as a white foam.

25 Example 38: 5'-O-Dimethoxytrityl-2'-C-Allyl-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (10)

5'-O-Dimethoxytrityl-2'-C-allyl-uridine (0.64 g, 1.12 mmol) was dissolved in dry dichloromethane under dry argon. N,N-Diisopropylethylamine (0.39 mL, 2.24 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (0.35 mL, 1.57 mmol) was added dropwise to  
30 the stirred reaction solution and stirring was continued for 2 h at RT. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture was concentrated *in vacuo* (40 °C)

and purified by flash chromatography on silica gel using a gradient of 10-60% EtOAc in hexanes containing 1% triethylamine mixture as eluent. Yield: 0.78 g (90%), white foam.

Example 39: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-C-Allyl-~~N~~<sup>4</sup>-Acetyl-Cytidine (11)

5 Triethylamine (6.35 mL, 45.55 mmol) was added dropwise to a stirred ice-cooled mixture of 1,2,4-triazole (5.66 g, 81.99 mmol) and phosphorous oxychloride (0.86 mL, 9.11 mmol) in 50 mL of anhydrous acetonitrile. To the resulting suspension a solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-  
10 2'-C-allyl uridine (2.32 g, 4.55 mmol) in 30 mL of acetonitrile was added dropwise and the reaction mixture was stirred for 4 h at room temperature. The reaction was concentrated *in vacuo* to a minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq. sodium bicarbonate and brine. The organic layer was dried over sodium  
15 sulfate and the solvent was removed *in vacuo*. The resulting foam was dissolved in 50 mL of 1,4-dioxane and treated with 29% aq. NH<sub>4</sub>OH overnight at room temperature. TLC (chloroform:methanol / 9:1) showed complete conversion of the starting material. The solution was evaporated, dried by coevaporation with anhydrous pyridine and acetylated with acetic anhydride  
20 (0.52 mL, 5.46 mmol) in pyridine overnight. The reaction mixture was quenched with methanol, evaporated, the residue was dissolved in chloroform, washed with sodium bicarbonate and brine. The organic layer was dried over sodium sulfate, evaporated to dryness and purified by flash chromatography on silica gel (3% MeOH in chloroform). Yield 2.3 g (90%) as  
25 a white foam.

Example 40: 5'-O-Dimethoxytrityl-2'-C-Allyl-~~N~~<sup>4</sup>-Acetyl-Cytidine

This compound was obtained analogously to the uridine derivative 9 in 55% yield.

Example 41: 5'-O-Dimethoxytrityl-2'-C-allyl-N<sup>4</sup>-Acetyl-Cytidine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (12)

2'-O-Dimethoxytrityl-2'-C-allyl-N<sup>4</sup>-acetyl cytidine (0.8 g, 1.31 mmol) was dissolved in dry dichloromethane under argon. N,N-Diisopropylethylamine  
5 (0.46 mL, 2.62 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (0.38 mL, 1.7 mmol) was added dropwise to a stirred reaction solution and stirring was continued for 2 h at room temperature. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture was  
10 concentrated *in vacuo* (40 °C) and purified by flash chromatography on silica gel using chloroform:ethanol / 98:2 with 2% triethylamine mixture as eluent. Yield: 0.91 g (85%), white foam.

Example 42: 2'-Deoxy-2'-Methylene-Uridine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine  
15 14 (Hansske,F.; Madej,D.; Robins,M. J. *Tetrahedron* 1984, 40, 125 and Matsuda,A.; Takenuki,K.; Tanaka,S.; Sasaki,T.; Ueda,T. *J. Med. Chem.* 19<sup>91</sup>, 34, 812) (2.2 g, 4.55 mmol ) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column.  
20 2'-Deoxy-2'-methylene-uridine (1.0 g, 3.3 mmol, 72.5%) was eluted with 20% MeOH in CH<sub>2</sub>Cl<sub>2</sub>.

Example 43: 5'-O-DMT-2'-Deoxy-2'-Methylene-Uridine (15)

2'-Deoxy-2'-methylene-uridine (0.91 g, 3.79 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in pyridine (10 mL) was added  
25 dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) and washed with sat. NaHCO<sub>3</sub>, water and brine. The organic extracts were dried over MgSO<sub>4</sub>, concentrated *in vacuo* and purified over a silica gel column  
30 using EtOAc:hexanes as eluant to yield 15 (0.43 g, 0.79 mmol, 22%).

Example 44: 5'-O-DMT-2'-Deoxy-2'-Methylene-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (17)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uracil (0.43 g, 0.8 mmol) dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.28 mL, 1.6 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.25 mL, 1.12 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.3 g, 0.4 mmol, 50%) was purified by flash column chromatography over silica gel using a 25-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R<sub>f</sub> 0.42 (CH<sub>2</sub>Cl<sub>2</sub>: MeOH / 15:1)

Example 45: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-Uridine

2'-Keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)uridine 14 (1.92 g, 12.6 mmol) and triphenylphosphine (2.5 g, 9.25 mmol) were dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A 60 °C solution of sodium chlorodifluoroacetate in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> and chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol, 70%) eluted with 25% hexanes in EtOAc.

Example 46: 2'-Deoxy-2'-Difluoromethylene-Uridine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol, 68%) was eluted with 20% MeOH in CH<sub>2</sub>Cl<sub>2</sub>.



**Example 47: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine (16)**

2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (1.42 g, 4.18 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) and washed with sat. NaHCO<sub>3</sub>, water and brine. The organic extracts were dried over MgSO<sub>4</sub>, concentrated *in vacuo* and purified over a silica gel column using 40% EtOAc:hexanes as eluant to yield 5'-O-DMT-2'-deoxy-2'-difluoromethylene-uridine 16 (1.05 g, 1.8 mmol, 45%).

**Example 48: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (18)**

1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uracil (0.577 g, 1 mmol) dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.36 mL, 2 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.44 mL, 1.4 mmol). The reaction mixture was stirred for 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.404 g, 0.52 mmol, 52%) was purified by flash chromatography over silica gel using 20-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R<sub>f</sub> 0.48 (CH<sub>2</sub>Cl<sub>2</sub>: MeOH / 15:1).

**Example 49: 2'-Deoxy-2'-Methylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-4-N-Acetyl-Cytidine 20**

Triethylamine (4.8 mL, 34 mmol) was added to a solution of POCl<sub>3</sub> (0.65 mL, 6.8 mmol) and 1,2,4-triazole (2.1 g, 30.6 mmol) in acetonitrile (20 mL) at 0 °C. A solution of 2'-deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl) uridine 19 (1.65 g, 3.4 mmol) in acetonitrile (20 mL) was added dropwise to the above reaction mixture and left to stir at room temperature for 4 h. The mixture was concentrated *in vacuo*, dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 x 100 mL) and washed with 5% NaHCO<sub>3</sub> (1 x 100 mL). The organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub> concentrated *in vacuo*, dissolved in dioxane (10 mL) and aq.

ammonia (20 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (3 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat. NaHCO<sub>3</sub> (5 mL). The mixture was concentrated *in vacuo*, dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 x 100 mL) and washed with 5% NaHCO<sub>3</sub> (1 x 100 mL). The organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-  
5 cytidine 20 (1.3 g, 2.5 mmol, 73%) was eluted with 20% EtOAc in hexanes.

10 Example 50: 1-(2'-Deoxy-2'-Methylene-5'-O-Dimethoxytrityl-β-D-ribofurano-  
syl)-4-N-Acetyl-Cytosine 21

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine 20 (1.3 g, 2.5 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The residue was  
15 triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-methylene-4-N-acetyl-cytidine (0.56 g, 1.99 mmol, 80%) was eluted with 10% MeOH in CH<sub>2</sub>Cl<sub>2</sub>. 2'-Deoxy-2'-methylene-4-N-acetyl-cytidine (0.56 g, 1.99 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (0.81 g, 2.4 mmol) in pyridine (10 mL) was added dropwise over 15 m. The  
20 resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) and washed with sat. NaHCO<sub>3</sub> (50 mL), water (50 mL) and brine (50 mL). The organic extracts were dried over MgSO<sub>4</sub>, concentrated *in vacuo* and purified over a silica gel column using  
25 EtOAc:hexanes / 60:40 as eluant to yield 21 (0.88 g, 1.5 mmol, 75%).

Example 51: 1-(2'-Deoxy-2'-Methylene-5'-O-Dimethoxytrityl-β-D-ribofurano-  
syl)-4-N-Acetyl-Cytosine 3'-(2-Cyanoethyl-N,N-diisopropylphosphoramidite)  
(22)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-4-N-acetyl-cytosine 21 (0.88 g, 1.5 mmol) dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was  
30 placed in a round-bottom flask under Ar. Diisopropylethylamine (0.8 mL, 4.5 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction mixture

was stirred 2 h at room temperature and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product 22 (0.82 g, 1.04 mmol, 69%) was purified by flash chromatography over silica gel using 50-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R<sub>f</sub> 0.36 (CH<sub>2</sub>Cl<sub>2</sub>:MeOH / 20:1).

Example 52: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropyl  
disiloxane-1,3-diyl)-4-N-Acetyl-Cytidine (24)

Et<sub>3</sub>N (6.9 mL, 50 mmol) was added to a solution of POCl<sub>3</sub> (0.94 mL, 10 mmol) and 1,2,4-triazole (3.1 g, 45 mmol) in acetonitrile (20 mL) at 0 °C. A solution of 2'-deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)uridine 23 ([described in example 45] 2.6 g, 5 mmol) in acetonitrile (20 mL) was added dropwise to the above reaction mixture and left to stir at RT for 4 h. The mixture was concentrated *in vacuo*, dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 x 100 mL) and washed with 5% NaHCO<sub>3</sub> (1 x 100 mL). The organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub> concentrated *in vacuo*, dissolved in dioxane (20 mL) and aq. ammonia (30 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (5 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat. NaHCO<sub>3</sub> (5mL). The mixture was concentrated *in vacuo*, dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 x 100 mL) and washed with 5% NaHCO<sub>3</sub> (1 x 100 mL). The organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine 24 (2.2 g, 3.9 mmol, 78%) was eluted with 20% EtOAc in hexanes.

Example 53: 1-(2'-Deoxy-2'-Difluoromethylene-5'-O-Dimethoxytrityl-β-D-ribofuranosyl)-4-N-Acetyl-Cytosine (25)

2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine 24 (2.2 g, 3.9 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-4-N-acetyl-cytidine (0.89 g, 2.8 mmol, 72%) was eluted with 10% MeOH in CH<sub>2</sub>Cl<sub>2</sub>. 2'-Deoxy-2'-difluoromethylene-

4-*N*-acetyl-cytidine (0.89 g, 2.8 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (1.03 g, 3.1 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) and washed with sat. NaHCO<sub>3</sub> (50 mL), water (50 mL) and brine (50 mL). The organic extracts were dried over MgSO<sub>4</sub>, concentrated *in vacuo* and purified over a silica gel column using EtOAc:hexanes / 60:40 as eluant to yield 25 (1.2 g, 1.9 mmol, 68%).

10 Example 54: 1-(2'-Deoxy-2'-Difluoromethylene-5'-O-Dimethoxytrityl-β-D-ribofuranosyl)-4-*N*-Acetylcytosine 3'-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite) (26)

15 1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-4-*N*-acetylcytosine 25 (0.6 g, 0.97 mmol) dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.5 mL, 2.9 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). The product 26, a white foam (0.52 g, 0.63 mmol, 65%) was purified by flash chromatography over silica gel using 30-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R<sub>f</sub> 0.48 (CH<sub>2</sub>Cl<sub>2</sub>:MeOH / 20:1).

Example 55: 2'-Keto-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine (28)

25 Acetic anhydride (4.6 mL) was added to a solution of 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine (Brown, J.; Christodolou, C.; Jones, S.; Modak, A.; Reese, C.; Sibanda, S.; Ubasawa A. *J. Chem. Soc. Perkin Trans. I* 1989, 1735) (6.2 g, 9.2 mmol) in DMSO (37 mL) and the resulting mixture was stirred at room temperature for 24 h. The mixture was concentrated *in vacuo*. The residue was taken up in EtOAc and washed with water. The organic layer was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The residue was purified on a silica gel column to yield 2'-keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine 28 (4.8 g, 7.2 mmol, 78%).

**Example 56: 2'-Deoxy-2'-methylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-Butylbenzoyl)-Adenosine (29)**

Under a pressure of argon, sec-butyllithium in hexanes (11.2 mL, 14.6 mmol) was added to a suspension of triphenylmethylphosphonium iodide (7.07 g, 17.5 mmol) in THF (25 mL) cooled at -78 °C. The homogeneous orange solution was allowed to warm to -30 °C and a solution of 2'-keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine 28 (4.87 g, 7.3 mmol) in THF (25 mL) was transferred to this mixture under argon pressure. After warming to RT, stirring was continued for 24 h. THF was evaporated and replaced by CH<sub>2</sub>Cl<sub>2</sub> (250 mL), water was added (20 mL), and the solution was neutralized with a cooled solution of 2% HCl. The organic layer was washed with H<sub>2</sub>O (20 mL), 5% aqueous NaHCO<sub>3</sub> (20 mL), H<sub>2</sub>O to neutrality, and brine (10 mL). After drying (Na<sub>2</sub>SO<sub>4</sub>), the solvent was evaporated *in vacuo* to give the crude compound, which was chromatographed on a silica gel column. Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine 29 (3.86 g, 5.8 mmol, 79%).

**Example 57: 2'-Deoxy-2'-Methylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine**

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine (3.86 g, 5.8 mmol) dissolved in THF (30 mL) was treated with 1 M TBAF in THF (15 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-methylene-6-N-(4-*t*-butylbenzoyl)-adenosine (1.8 g, 4.3 mmol, 74%) was eluted with 10% MeOH in CH<sub>2</sub>Cl<sub>2</sub>.

**Example 58: 5'-O-DMT-2'-Deoxy-2'-Methylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine (29)**

2'-Deoxy-2'-methylene-6-N-(4-*t*-butylbenzoyl)-adenosine (0.75 g, 1.77 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (0.66 g, 1.98 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in

CH<sub>2</sub>Cl<sub>2</sub> (100 mL) and washed with sat. NaHCO<sub>3</sub>, water and brin . The organic extracts w re dried over MgSO<sub>4</sub>, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as an eluant to yield 29 (0.81 g, 1.1 mmol, 62%).

5 Example 59: 5'-O-DMT-2'-Deoxy-2'-Methylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine 3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (31)

1- (2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-6-N-(4-*t*-butylbenzoyl)-adenine 29 dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was placed in a round bottom flask under Ar. Diisopropylethylamine was added, followed by  
10 the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite. The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). The product was purified by flash chromatography over silica gel using 30-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant (0.7 g, 0.76  
15 mmol, 68%). R<sub>f</sub> 0.45 (CH<sub>2</sub>Cl<sub>2</sub>: MeOH / 20:1)

Example 60: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-Butylbenzoyl)-Adenosine

2'-Keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine 28 (6.7 g, 10 mmol) and triphenylphosphine (2.9 g, 11 mmol ) were  
20 dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A warm (60 °C) solution of sodium chlorodifluoroacetate (2.3 g, 15 mmol) in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> and  
25 chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine (4.1g, 6.4 mmol, 64%) eluted with 15% hexanes in EtOAc.

Example 61: 2'-Deoxy-2'-Difluoromethylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine

30 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine (4.1 g, 6.4 mmol) dissolved in THF (20 mL)

was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-6-*N*-(4-*t*-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol, 77%) was eluted with 20% MeOH in CH<sub>2</sub>Cl<sub>2</sub>.

5 Example 62: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine (30)

2'-Deoxy-2'-difluoromethylene-6-*N*-(4-*t*-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. 10 The mixture was concentrated *in vacuo* and the residue taken up in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) and washed with sat. NaHCO<sub>3</sub>, water and brine. The organic extracts were dried over MgSO<sub>4</sub>, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as eluant to yield 30 (2.6 g, 3.41 15 mmol, 69%).

Example 63: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine 3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (32)

1-(2'-Deoxy-2'-difluoromethylene-5'-*O*-dimethoxytrityl-β-D-ribofuranosyl)-6-*N*-(4-*t*-butylbenzoyl)-adenine 30 (2.6 g, 3.4 mmol) dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> 20 (25 mL) was placed in a round bottom flask under Ar. Diisopropylethylamine (1.2 mL, 6.8 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (1.06 mL, 4.76 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). 32 (2.3 g, 2.4 mmol, 25 70%) was purified by flash column chromatography over silica gel using 20-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R<sub>f</sub> 0.52 (CH<sub>2</sub>Cl<sub>2</sub>: MeOH / 15:1).

Example 64: 2'-Deoxy-2'-Methoxycarbonylmethylidene-3',5'-*O*-(Tetraisopropylidisiloxane-1,3-diyl)-Uridine (33)

30 Methyl(triphenylphosphoranylidene)acetate (5.4 g, 16 mmol) was added to a solution of 2'-keto-3',5'-*O*-(tetraisopropyl disiloxane-1,3-diyl)-uridine 14 in

CH<sub>2</sub>Cl<sub>2</sub> und r argon. Th mixture was left to stir at RT for 30 h. CH<sub>2</sub>Cl<sub>2</sub> (100 mL) and water were added (20 mL), and the solution was neutralized with a cooled solution of 2% HCl. The organic layer was washed with H<sub>2</sub>O (20 mL), 5% aq. NaHCO<sub>3</sub> (20 mL), H<sub>2</sub>O to neutrality, and brine (10 mL). After drying (Na<sub>2</sub>SO<sub>4</sub>), the solvent was evaporated *in vacuo* to give crude product, that was chromatographed on a silica gel column. Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methoxycarbonylmethylidene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine **33** (5.8 g, 10.8 mmol, 67.5%).

Example 65: 2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine (34)

Et<sub>3</sub>N•3 HF (3 mL) was added to a solution of 2'-deoxy-2'-methoxycarbonylmethylidene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine **33** (5 g, 9.3 mmol) dissolved in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) and Et<sub>3</sub>N (15 mL). The resulting mixture was evaporated *in vacuo* after 1 h and chromatographed on a silica gel column eluting 2'-deoxy-2'-methoxycarbonylmethylidene-uridine **34** (2.4 g, 8 mmol, 86%) with THF:CH<sub>2</sub>Cl<sub>2</sub> / 4:1.

Example 66: 5'-O-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine (35)

2'-Deoxy-2'-methoxycarbonylmethylidene-uridine **34** (1.2 g, 4.02 mmol) was dissolved in pyridine (20 mL). A solution of DMT-Cl (1.5 g, 4.42 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) and washed with sat. NaHCO<sub>3</sub>, water and brine. The organic extracts were dried over MgSO<sub>4</sub>, concentrated *in vacuo* and purified over a silica gel column using 2-5% MeOH in CH<sub>2</sub>Cl<sub>2</sub> as an eluant to yield 5'-O-DMT-2'-deoxy-2'-methoxycarbonylmethylidene-uridine **35** (2.03 g, 3.46 mmol, 86%).

Example 67: 5'-O-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine 3'-(2-cyanoethyl)-N,N-diisopropylphosphoramidite (36)

1-(2'-Deoxy-2'-2'-methoxycarbonylmethylidene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uridine **35** (2.0 g, 3.4 mmol) dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (1.2 mL,



6.8 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.91 mL, 4.08 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). 5'-*O*-DMT-2'-deoxy-2'-methoxycarbonylmethylidene-uridine 3'-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite) **36** (1.8 g, 2.3 mmol, 67%) was purified by flash column chromatography over silica gel using a 30-60% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. *R*<sub>f</sub> 0.44 (CH<sub>2</sub>Cl<sub>2</sub>:MeOH / 9.5:0.5).

Example 68: 2'-Deoxy-2'-Carboxymethylidene-3',5'-*O*-(Tetraisopropylidisiloxane-1,3-diyl)-Uridine **37**

2'-Deoxy-2'-methoxycarbonylmethylidene-3',5'-*O*-(tetraisopropylidisiloxane-1,3-diyl)-uridine **33** (5.0 g, 10.8 mmol) was dissolved in MeOH (50 mL) and 1 N NaOH solution (50 mL) was added to the stirred solution at RT. The mixture was stirred for 2 h and MeOH removed *in vacuo*. The pH of the aqueous layer was adjusted to 4.5 with 1N HCl solution, extracted with EtOAc (2 x 100 mL), washed with brine, dried over MgSO<sub>4</sub> and concentrated *in vacuo* to yield the crude acid. 2'-Deoxy-2'-carboxymethylidene-3',5'-*O*-(tetraisopropylidisiloxane-1,3-diyl)-uridine **37** (4.2 g, 7.8 mmol, 73%) was purified on a silica gel column using a gradient of 10-15% MeOH in CH<sub>2</sub>Cl<sub>2</sub>.

Example 69: Synthesis of 2'-*C*-allyl-U phosphoramidite from 5'-*O*-DMT-3'-*O*-TBDMS-Uridine

Referring to Figure 54, in order to simplify the synthetic scheme for phosphoramidites **5** and **8** we also explored the potential of 5'-*O*-DMT-3'-*O*-TBDMS-Uridine **10** (side product in preparation of standard RNA monomers) as a starting material in the synthesis of key intermediate **4**. Phenoxythiocarbonylation of starting synthon **10** according to Robins (Robins, M. J., Wilson J. S. and Hansske, F. (1983), *J. Am. Chem. Soc.*, 105, 4059) surprisingly led to thioester **11** ( 91 %) without noticeable migration (Scaringe, S.A., Franclyn, C. & Usman, N. (1990) *Nucleic Acids Res.*, 18, 5433-5441) of the TBDMS group. Comparative analysis of <sup>1</sup>H NMR data for compounds **10** and **11** revealed that resonance of H-2' experienced up field shift of 2.0 ppm (from 6.06 to 4.13) in **11** compare to starting compound **10**, at the same time chemical shift of H-3' and H-1' changed only slightly: 4.83 ppm(H-3') and

6.48 ppm (H-1') in 11 compare to 4.36(H-3') ppm and 5.93 ppm (H-1') in 10 and chemical shift of H-4' remains practically unchanged indicating acylation at C-2-OH. Heck allylation of intermediate 11 with 2,2'-Azobis-(2-methyl propionitrile) (other groups can be introduced by standard procedures) resulted in a formation of 2'-C-allyl derivative 12 (70 %) and related 2'-deoxy by-product (15%). Subsequent desilylation of 12 led to 5'-O-DMT derivative 4 identical to the one synthesized from thioester 2. Since the starting material for this route is commercially available this may represent a less laborious way to key synthon 4 as well as for other 2'- modified monomers. This methodology can be used to introduce other 2'-C-allyl groups using compound 11 (or its equivalent for other bases) as an intermediate.

Example 70: Synthesis of 5'-O-Dimethoxytrityl-2'-O-Phenoxythiocarbonyl-3'-O-t-butylidimethylsilyl-uridine 11.

To a stirred solution of 5'-O-Dimethoxytrityl-3'-O-t-butylidimethylsilyl-uridine (Commercially available from Chem Genes Corporation) (5.0 g 7.57 mmol) and dimethylaminopyridine (1.8g, 15 mmol) in 100 ml of dry acetonitrile a solution of phenylchlorothionoformate (1.26ml, 9.1 mmol) in 25 ml of acetonitrile was added dropwise and the reaction mixture stirred at room temperature for 3 hours. TLC (ethylacetate-hexanes 1:1) showed disappearance of starting material and the reaction mixture was concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel CH<sub>2</sub>Cl<sub>2</sub> as an eluent to give 5.51g (91.3%) of the product.

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.95 (s, 9H, tBu), 0.11 (s, 3H, CH<sub>3</sub>), 0.04 (s, 3H, CH<sub>3</sub>) 3.57 (2H, H5', H5'', m J5',4'=2.4., J5'',4'=2.8., J5',5''=11.0), 3.86 (6H, OCH<sub>3</sub>, s), 4.07 (1H, H4', m), 4.83 (1H, H3', dd, J3',4'=2.8 J3',2'=5.2), 5.44 (1H, H5, d, J5,6=8.0 ) 5.99 (1H, H2', dd, J2',1'=6.4 , J2',3'= 5.2 ), 6.46 (1H, H1', d, J1',2'=6.4) , 6.89-7.79 (18H, DMT, Phe, m), 7.88 (1H, H6, d, J6,5=8.0), 7.95 (1H, N-H, bs).

Example 71: Synthesis of 5'-O-Dimethoxytrityl-2'-C-Allyl-3'-O-t-butylidimethylsilyl-uridine(12)

To a refluxing under argon solution of 5'-O-Dimethoxytrityl-2'-O-Phenoxythiocarbonyl-3'-O-t-butylidimethylsilyl-uridine (5.5g, 6.9 mmol) and

allyltributyltin (10.7ml, 34.5 mmol) in dry toluene (150 ml) a solution of 2',2'-Azobis-(2-methyl propionitrile) (0.28g 1.72 mmol) in 50 ml of dry toluene was added dropwise for 1 hour. The resulting mixture was allowed to reflux under argon for additional 2 hours. After that it was concentrated in vacuo and  
 5 purified by flash chromatography on silica gel with gradient ethylacetate in hexanes (0-30%) as an eluent. Yield 3.38g (70.0%).

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.95 (s, 9H, tBu), 0.11 (s, 3H, CH<sub>3</sub>), 0.04 (s, 3H, CH<sub>3</sub>), 2.23 (1H, H6', m), 2.38-2.52 (2H, H6" and H2', m), 3.46 (2H, H5' and H5", m, J<sub>5',4'</sub>=2.5, J<sub>5'',4'</sub>=3.2 J<sub>5',5''</sub>=10.8), 3.86 (6H, OCH<sub>3</sub>, s), 4.13 (1H, H4', dd, J<sub>4',3'</sub>=8.0, J<sub>4',5'</sub>=3.2, J<sub>4',5''</sub>=2.5), 4.46 (1H, H3', m), 5.15 (1H, H8', d, J<sub>8',7'</sub>=10.0), 5.20 (1H, H9', d, J<sub>9',7'</sub>=17.3), 5.44 (1H, H5, d, J<sub>5,6</sub>=8.0), 5.81 (1H, H7', dddd, J<sub>7',6'</sub>=6.0, J<sub>7',6''</sub>=8.0), 6.14 (1H, H1', d, J<sub>1',2'</sub>=8.0), 6.88-7.52 (13H, DMT, m), 7.76 (1H, H6, d, J<sub>6,5</sub>=8.0), 8.17 (1H, N-H, bs)

Example 72: Synthesis of 5'-O-Dimethoxytrityl-2'-C-Allyl Uridine (4) from 5'-O-Dimethoxytrityl-2'-C-Allyl-3'-O-t-butylidimethyl-silyl-uridine (12).  
 15

Standard deprotection of TBDMS derivative 12 utilizing general method A furnished product 4 (yield 80%) identical to the compound prepared from 2'-C-allyl derivative 3.

### Uses

20 The alkyl substituted nucleotides of this invention can be used to form stable oligonucleotides as discussed above for use in enzymatic cleavage or antisense situations. Such oligonucleotides can be formed enzymatically using triphosphate forms by standard procedure. Administration of such oligonucleotides is by standard procedure. See Sullivan *et al.* PCT WO  
 25 94/02595.

The following are non-limiting examples showing the synthesis of nucleic acids using 2'-O-methylthioalkyl-substituted phosphoramidites and the syntheses of the amidites.

Example 73: Synthesis of Hammerhead Ribozymes Containing 2'-O-alkylthioalkylnucleotides & Other Modified Nucleotides

The method of synthesis follows the procedure for normal RNA synthesis as described in Usman, N.; Ogilvie, K.K.; Jiang, M.-Y.; Cedergren, R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe, S.A.; Franklyn, C.; Usman, N. *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. These 2'-O-alkylthioalkyl substituted phosphoramidites may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group I or Group II intron catalytic nucleic acids, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 74: Synthesis of base-protected 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl) nucleosides (2)

Referring to Figure 55, standard introduction of "Markiewicz" protecting group to the base-protected nucleosides according to "Oligonucleotides and Analogues. A Practical Approach", ed. F. Eckstein, IRL Press, 1991 resulted in protected nucleosides (2) with 85-100% yields. Briefly, in a non-limiting example, Uridine (20g, 81.9 mmol) was dried by two coevaporations with anhydrous pyridine and re dissolved in the anhydrous pyridine. The above solution was cooled (0°C) and solution of 1,3-dichloro-1,1,3,3-tetraisopropylsiloxane (28.82 mL, 90.09 mmol) in 30 mL of anhydrous dichloroethane was added dropwise under stirring. After the addition was completed the reaction mixture was allowed to warm to room temperature and stirred for additional two hours. Then it was quenched with MeOH (25 mL) and evaporated to dryness. The residue was dissolved in methylene chloride and washed with saturated NaHCO<sub>3</sub> and brine. The organic layer was evaporated to dryness and then coevaporated with toluene to remove traces of pyridine to give 39g (98%) of compound 2 (B=Ura) which was used without further purification.

Other 3',5'-O-(tetraisopropylidisiloxane-1,3-di-yl)- nucleosides were obtained in 75-90% yields, using the protocol described above, starting from

base-protected nucleosides with final purification of the products by flash chromatography on silica gel when necessary.

Example 75: General procedure for the synthesis of 2'-O-methylthiomethyl nucleosides (3)

- 5 Referring to Figure 55, to a stirred ice-cooled solution of the mixture of base-protected 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl) nucleoside (2) (7 mmol), methyl disulfide (70 mmol), 2,6-lutidine (7 mmol) in methylene chloride (100 mL) or mixture methylene chloride - acetonitrile (1:1) under positive pressure of argon, solution of benzoyl peroxide (28 mmol) in methylene
- 10 chloride was added dropwise during 1 hour. After complete addition the reaction mixture was stirred at 0°C under argon for additional 1 hour. The solution was allowed to warm to room temperature, diluted with methylene chloride (100 mL), washed twice with saturated aq NaHCO<sub>3</sub> and brine. The organic layer was dried over sodium sulfate and evaporated to dryness. The
- 15 residue was purified by flash chromatography on silica using 1-2% methanol in methylene chloride as an eluent to give corresponding methylthiomethyl nucleosides with 55% yield.

Example 76: 5'-O-Dimethoxytrityl-2'-O-Methylthiomethyl-Nucleosides. (6)

- Method A. The solution of the base-protected 3',5'-O-
- 20 (tetraisopropylidisiloxane-1,3-diyl)-2'-O-methylthiomethyl nucleoside (3) (2.00 mmol) in 10 ml of dry tetrahydrofuran (THF) was treated with 1M solution of tetrabutylammoniumfluoride in THF (3.0 ml) for 10-15 minutes at room temperature. Resulting mixture was evaporated, the residue was loaded to the silica gel column, washed with 1L of chloroform, and the desired
- 25 deprotected compound was eluted with 5-10% methanol in dichloromethane. Appropriate fractions were combined, solvents removed by evaporation, and the residue was dried by coevaporation with dry pyridine. The oily residue was redissolved in dry pyridine, dimethoxytritylchloride (1.2 eq) was added and the reaction mixture was left under anhydrous conditions overnight. The
- 30 reaction was quenched with methanol (20 ml), evaporated, dissolved in chloroform, washed with saturated aq sodium bicarbonate and brine. Organic layer was dried over sodium sulfate and evaporated. The residue was purified

by flash chromatography on silica gel to give 5'-O-Dimethoxytrityl derivatives with 70-80% yield.

**Method B.** Alternatively, 5'-O-Dimethoxytrityl-2'-O-Methylthiomethyl-Nucleosides (6) may also be synthesized using 5'-O-Dimethoxytrityl-3'-O-t-Butyl-dimethylsilyl Nucleosides (4) as the starting material. Compound 4 is commercially available as a by-product during RNA phosphoramidite synthesis. Compound 4 is converted in to 3'-O-t-butyl dimethylsilyl-2'-O-methylthiomethyl nucleoside 5, as described under example 3. The solution of the base-protected 3'-O-t-butyl dimethylsilyl-2'-O-methylthiomethyl nucleoside 5 (2.00 mmol) in 10 ml of dry tetrahydrofuran (THF) was treated with 1M solution of tetrabutylammoniumfluoride in THF (3.0 ml) for 10-15 minutes at room temperature. The resulting mixture was evaporated, and purified by flash silica gel chromatography to give nucleosides 6 in 90% yield.

Example 77: 5'-O-Dimethoxytrityl-2'-O-Methylthiomethyl-Nucleosides-3'-(2-Cyanoethyl-N,N-diisopropylphosphoramidites) (7)

Standard phosphorylation of nucleoside 6 according to Scaringe, S.A.; Franklin, C.; Usman, N. *Nucleic Acids Res.* 1990, 18, 5433-5441 yielded phosphoramidites in 70-85% yield.

Example 78: General procedure for the synthesis of 2'-O-Methylthiophenyl nucleosides.

To a stirred ice-cooled solution of the mixture of base-protected 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl) nucleoside (14.7 mmol), thioanisole (147 mmol), N,N-dimethylaminopyridine (58.8 mmol) in acetonitrile (100 mL) under positive pressure of argon, benzoyl peroxide (36.75 mmol) was added portionwise over 3 hours. After complete addition the reaction mixture was allowed to warm to room temperature and was stirred under argon for an additional 1 hour. The solvents were removed in vacuo, the residue was dissolved in ethylacetate, washed twice with saturated aq NaHCO<sub>3</sub> and brine. The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by flash chromatography on silica using mixture EtOAc-hexanes (1:1) as eluent to give the corresponding methylthiophenyl nucleosides with 55-65% yield.

Example 79: 5'-O-Dimethoxytrityl-2'-O-Methylthiophenyl-Nucleosides.

These compounds were prepared as described above under examples 76 and 76.

5 Example 80: 5'-O-Dimethoxytrityl-2'-O-Methylthiophenyl-Nucleosides-3'-(2-Cyanoethyl N,N-diisopropylphosphoramidites)

Standard phosphitylation according to Scaringe, S.A.; Franklyn, C.; Usman, N. *Nucleic Acids Res.* 1990, 18, 5433-5441 yielded phosphoramidites in 70-85% yield.

Example 81: Ribozymes containing 2'-O-methylthiomethyl substitutions

10 In a non-limiting example 2'-O-methylthioalkyl substitutions were made at various positions within a hammerhead ribozyme motif (Fig. 56, including U4 and U7 positions). The target site B was targeted by the hammerhead ribozyme in this non-limiting example.

15 Hammerhead ribozymes (see Fig. 56) were synthesized using solid-phase synthesis, as described above. Several positions were modified, individually or in combination, with 2'-O-methylthiomethyl groups.

RNA cleavage assay *in vitro*:

20 Substrate RNA is 5' end-labeled using [ $\gamma$ - $^{32}$ P] ATP and T4 polynucleotide kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace amount ( $\leq 1$  nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme are denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10 -15 min. The ribozyme and substrate are incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl and 10 mM MgCl<sub>2</sub>. The reaction is  
25 initiated by mixing the ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5  $\mu$ l are taken at regular intervals of time and the reaction is quenched by mixing with equal volume of 2X formamide stop mix. The samples are resolved on 20 % denaturing polyacrylamide gels. The results are quantified and percentage of target RNA cleaved is plotted as a function of  
30 time.

Referring to Figure 57, hammerhead ribozymes containing 2'-O-methylthiomethyl modifications at various positions cleave the target RNA efficiently. Surprisingly, all the 2'-O-methylthiomethyl -substituted ribozymes cleaved the target RNA more efficiently compared to the control hammerhead  
5 ribozyme.

Sequences listed in Figure 56 and the modifications described in Figure 56 and 57 are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions, mutations, chemical modifications) of the ribozyme and RNA containing other  
10 combinations of 2'-hydroxyl group modifications can be readily generated using techniques known in the art, and are within the scope of the present invention.

The following are non-limiting examples showing the synthesis of non-nucleotide mimetic-containing catalytic nucleic acids using non-nucleotide  
15 phosphoramidites.

Such non-nucleotides can be located in the binding arms, core or the loop adjacent stem II of a hammerhead type ribozyme. Those in the art following the teachings herein can determine optimal locations in these regions. Surprisingly, abasic moieties can be located in the core of such a  
20 ribozyme.

#### Example 82: Synthesis of Abasic nucleotides

The synthesis of 1-deoxy-D-ribofuranose phosphoramidite 9 is shown in Figure 58. Our initial efforts concentrated on the deoxygenation of synthon 1, prepared by a "one pot" procedure from D-ribose. Phenoxythiocarbonylation  
25 of acetonide 1 under Robins conditions led to the  $\beta$ -anomer 2 ( $J_{1,2} = 1.2$  Hz) in modest yield (45-55%). Radical deoxygenation using  $\text{Bu}_3\text{SnH/AIBN}$  resulted in the formation of the ribitol derivative 3 in 50% yield. Subsequent deprotection with 90%  $\text{CF}_3\text{COOH}$  (10 m) and introduction of a dimethoxytrityl group led to the key intermediate 4 in 40% yield (Yang et al., *Biochemistry*  
30 1992, 31, 5005-5009; Perreault et al., *Biochemistry* 1991, 30, 4020-4025; Paoletta et al., *EMBO J.* 1992, 11, 1913-1919; Peiken et al., *Science* 1991, 253, 314-317).



The low overall yield of this route prompted us to investigate a different approach to 4 (Fig. 58). Phenylthioglycosides, successfully employed in the Keck reaction, appeared to be an alternative. However, it is known that free-radical reduction of the corresponding glycosyl bromides with participating acyl groups at the C2-position can result in the migration of the 2-acyl group to the C1-position (depending on  $\text{Bu}_3\text{SnH}$  concentration). Therefore we subjected phenylthioglycoside 5 to radical reduction with  $\text{Bu}_3\text{SnH}$  (6.1 eq.) in the presence of  $\text{Bz}_2\text{O}_2$  (2 eq.) resulting in the isolation of tribenzoate 6 in 63% yield (Fig. 9B). Subsequent debenzoylation and dimethoxytritylation led to synthon 4 in 70% yield. Introduction of the TBDMS group, using standard conditions, resulted in the formation of a 4:1 ratio of 2- and 3-isomers 8 and 7. The two regioisomers were separated by silica gel chromatography. The 2-*O*-*t*-butyldimethylsilyl derivative 8 was phosphitylated to provide phosphoramidite 9 in 82% yield.

15 Example 83: RNA cleavage assay *in vitro*

Ribozymes and substrate RNAs were synthesized as described above. Substrate RNA was 5' end-labeled using [ $\gamma$ - $^{32}\text{P}$ ] ATP and T4 polynucleotide kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace amount ( $\leq 1$  nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme were denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10 -15 min. The ribozyme and substrate were incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl and 10 mM  $\text{MgCl}_2$ . The reaction was initiated by mixing the ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5  $\mu\text{l}$  are taken at regular intervals of time and the reaction quenched by mixing with an equal volume of 2X formamide stop mix. The samples were resolved on 20 % denaturing polyacrylamide gels. The results were quantified and percentage of target RNA cleaved is plotted as a function of time.

30 Referring to Figure 59 there is shown the general structure of a hammerhead ribozyme targeted against site B (HH-B) with various bases numbered. Various substitutions were made at several of the nucleotide positions in HH-B. Specifically referring to Figure 60, substitutions were made

at the U4 and U7 positions marked as X4 and X7 and also in loop II in the positions marked by an X. The RNA cleavage activity of these substituted ribozymes is shown in the following figures. Specifically, Figure 61 shows cleavage by an abasic substituted U4 and an abasic substituted U7. As will  
5 be noted, abasic substitution at U4 or U7 does not significantly affect cleavage activity. In addition, inclusion of all abasic moieties in stem II loop does not significantly reduce enzymatic activity as shown in Figure 62. Further, inclusion of a 3' inverted deoxyribose does not inactivate the RNA cleavage activity as shown in Figure 63.

10 Example 84: Smooth Muscle Cell Proliferation Assay

Hammerhead ribozyme (HH-A) is targeted to a unique site (site A) within *c-myc* mRNA. Expression of *c-myc* protein has been shown to be essential for the proliferation of rat smooth muscle cell (Brown et al., 1992 *J. Biol. Chem.* 267, 4625).

15 The ribozymes that cleaved site A within *c-myc* RNA described above were assayed for their effect on smooth muscle cell proliferation. Rat vascular smooth muscle cells were isolated and cultured as described (Stinchcomb et al., *supra*). These primary rat aortic smooth muscle cells (RASMC) were plated in a 24-well plate ( $5 \times 10^3$  cells/well) and incubated at 37°C in the  
20 presence of Dulbecco's Minimal Essential Media (DMEM) and 10% serum for ~16 hours.

These cells were serum-starved for 48-72 hours in DMEM (containing 0.5% serum) at 37°C. Following serum-starvation, the cells were treated with lipofectamine (LFA)-complexed ribozymes (100 nM ribozyme was complexed  
25 with LFA such that LFA:ribozyme charge ration is 4:1).

Ribozyme:LFA complex was incubated with serum-starved RASMC cells for four hours at 37°C. Following the removal of ribozyme:LFA complex from cells (after 4 hours), 10% serum was added to stimulate smooth cell proliferation. Bromo-deoxyuridine (BrdU) was added to stain the cells. The  
30 cells were stimulated with serum for 24 hours at 37°C.

Following serum-stimulation, RASMC cells were quenched with hydrogen peroxide (0.3% H<sub>2</sub>O<sub>2</sub> in methanol) for 30 min at 4°C. The cells were then denatured with 0.5 ml 2N HCl for 20 min at room temperature. Horse serum (0.5 ml) was used to block the cells at 4°C for 30 min up to -16  
5 hours.

The RASMC cells were stained first by treating the cells with anti-BrdU (primary) antibody at room temperature for 60 min. The cells were washed with phosphate-buffered saline (PBS) and stained with biotinylated affinity-purified anti-mouse IgM (Pierce, USA) secondary antibody. The cells were  
10 counterstained using avidin-biotinylated enzyme complex (ABC) kit (Pierce, USA).

The ratio of proliferating:non-proliferating cells was determined by counting stained cells under a microscope. Proliferating RASMCs will incorporate BrdU and will stain brown. Non-proliferating cells do not  
15 incorporate BrdU and will stain purple.

Referring to Figure 64 there is shown a ribozyme which cleaves the site A referred to as HH-A. Substitutions of abasic moieties in place of U4 as shown in Figure 65 provided active ribozyme as shown in Figure 66 using the above-noted rat aortic smooth muscle cell proliferation assay.

20 The method of this invention generally features HPLC purification of ribozymes. An example of such purification is provided below in which a synthetic ribozyme produced on a solid phase is blocked. This material is then released from the solid phase by a treatment with methanolic ammonia, subsequently treated with tetrabutylammonium fluoride, and purified on  
25 reverse phase HPLC to remove partially blocked ribozyme from "failure" sequences. Such "failure" sequences are RNA molecules which have a nucleotide base sequence shorter to that of the desired enzymatic RNA molecule by one or more of the desired bases in a random manner, and possess free terminal 5'-hydroxyl group. This terminal 5'-hydroxyl in a  
30 ribozyme with the correct sequence is still blocked by lipophilic dimethoxytrityl group. After such partially blocked enzymatic RNA is purified, it is deblocked by a standard procedure, and passed over the same or a similar HPLC

reverse phase column to remove other contaminating components, such as other RNA molecules or nucleotides or other molecules produced in the deblocking and synthetic procedures. The resulting molecule is the native enzymatically active ribozyme in a highly purified form.

- 5        Below are provided examples of such a method. These examples can be readily scaled up to allow production and purification of gram or even kilogram quantities of ribozymes.

Example 85: HPLC Purification, Reverse-Phase

- 10        In this example solid phase phosphoramidite chemistry was employed for synthesis of a ribozyme. Monomers used were 2'-*t*-butyl-dimethylsilyl cyanoethylphosphoramidites of uridine, *N*-benzoyl-cytosine, *N*-phenoxyacetyl adenosine, and guanosine (Glen Research, Sterling, VA).

- 15        Solid phase synthesis was carried out on either an ABI 394 or 380B DNA/RNA synthesizer using the standard protocol provided with each machine. The only exception was that the coupling step was increased from 10 to 12 minutes. The phosphoramidite concentration was 0.1 M. Synthesis was done on a 1  $\mu$ mol scale using a 1  $\mu$ mol RNA reaction column (Glen Research). The average coupling efficiencies were between 97% and 98% for the 394 model and between 97% and 99% for the 380B model, as  
20        determined by a calorimetric measurement of the released trityl cation. The final 5'-DMT group was not removed.

- 25        After synthesis, the ribozymes were cleaved from the CPG support, and the base and phosphotriester moieties were deprotected in a sterile vial by incubation in dry ethanolic ammonia (2 mL) at 55 °C for 16 hours. The reaction mixture was cooled on dry ice. Later, the cold liquid was transferred  
into a sterile screw cap vial and lyophilized.

- 30        To remove the 2'-*t*-butyldimethylsilyl groups from the ribozyme the obtained residue was suspended in 1 M tetra-*n*-butylammonium fluoride in dry THF (TBAF), using a 20-fold excess of the reagent for every silyl group, for 16 hours at ambient temperature. The reaction was quenched by adding an

equal volume of a sterile 1 M triethylamine acetate, pH 6.5. The sample was cooled and concentrated on a SpeedVac to half of the initial volume.

The ribozymes were purified in two steps by HPLC on a C4 300 Å 5 µm DeltaPak column in an acetonitrile gradient.

- 5        The first step, or "trityl on" step, was a separation of 5'-DMT-protected ribozyme(s) from failure sequences lacking a 5'-DMT group. Solvents used for this step were: A (0.1 M triethylammonium acetate, pH 6.8) and B (acetonitrile). The elution profile was: 20% B for 10 minutes, followed by a linear gradient of 20% B to 50% B over 50 minutes, 50% B for 10 minutes, a linear gradient of 50% B to 100% B over 10 minutes, and a linear gradient of 100% B to 0% B over 10 minutes.
- 10

- 15        The second step was a purification of a completely deprotected, *i.e.* following the removal of the 5'-DMT group, ribozyme by a treatment with 2% trifluoroacetic acid or 80% acetic acid on a C4 300 Å 5 µm DeltaPak column in an acetonitrile gradient. Solvents used for this second step were: A (0.1 M Triethylammonium acetate, pH 6.8) and B (80% acetonitrile, 0.1 M triethylammonium acetate, pH 6.8). The elution profile was: 5% B for 5 minutes, a linear gradient of 5% B to 15% B over 60 minutes, 15% B for 10 minutes, and a linear gradient of 15% B to 0% B over 10 minutes.

- 20        The fraction containing ribozyme, which is in the triethylammonium salt form, was cooled and lyophilized on a SpeedVac. Solid residue was dissolved in a minimal amount of ethanol and ribozyme in sodium salt form was precipitated by addition of sodium perchlorate in acetone. ( $K^+$  or  $Mg^{2+}$  salts can be produced in an equivalent manner.) The ribozyme was collected
- 25        by centrifugation, washed three times with acetone, and lyophilized.

Example 86: RNA and Ribozyme Deprotection of Exocyclic Amino Protecting Groups Using ethylamine (EA)

- 30        The polymer-bound oligonucleotide, either trityl-on or off, was suspended in a solution of ethylamine (EA) @ 25-55 °C for 10-30 min to remove the exocyclic amino protecting groups (see Figure 67). The supernatant was removed from the polymer support. The support was washed with 1.0 mL of

EtOH:MeCN:H<sub>2</sub>O/3:1:1, vortexed and the supernatant was then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, were dried to a white powder.

5 Table EVII is a summary of the results obtained using the improvements outlined in this application for base deprotection. From this data it is evident EA at 55° for 10 m or 40° for 10 m is efficient. The HPLC peak structure is almost identical between these schemes, and the yield for the ethylamine deprotected oligos is actually slightly better than the methylamine.

10 The second step of the deprotection of RNA molecules may be accomplished by removal of the 2'-hydroxyl alkylsilyl protecting group using TBAF for 8-24 h (Usman *et al. J. Am. Chem. Soc.* 1987, 109, 7845-7854). Applicant has determined that the use of anhydrous TEA•HF in *N*-methylpyrrolidine (NMP) for 0.5-1.5 h @ 55-65 °C gives equivalent or better results.

15 The following are examples of preferred embodiments of the present invention. Those in the art will recognize that these are not limiting examples but rather are provided to guide those in the art to the full breadth of meaning of the present invention. Routine procedures can be used to utilize other coupling regions not exemplified below.

20 Ribozymes were synthesized in two parts and tested without ligation for catalytic activity. Referring to Fig. 72, the cleavage activity of the half ribozymes containing between 5 and 8 base pairs stem IIs at 40 nM under single turnover conditions was comparable to that of the full length oligomer as shown in Figs. 73 and 74. The same half ribozymes were synthesized with  
25 suitable modifications at the nascent stem II loop to allow for crosslinking. The halves were purified and chemically ligated, using a variety of crosslinking methods. The resulting full length ribozymes (see Fig. 71) exhibited similar cleavage activity as the linearly synthesized full length oligomer as shown in Fig. 74.

**Example 87**

Referring to Fig. 70 the 5' half of a hammerhead ribozyme was provided with a ribose group. This was oxidatively cleaved with  $\text{NaIO}_4$  and reacted with the 3' half of the ribozyme having an amino group under reducing conditions.

- 5 The resulting ribozyme consisted of the two half ribozyme linked by a morpholino group.

One equivalent of (200 micrograms) of 5' half hammerhead with a 3'OH and 5 equivalents (1000 micrograms) of 3' half with 5' C5-NH<sub>2</sub> all with HH-A were used in this reaction. The limiting oligonucleotide was oxidized first with  
10 3.6 equivalents of sodium periodate for sixty minutes on ice in DEPC water quenched with 7.2 equivalents of ethylene glycol for 30 minutes on ice and the 5 equivalents of the amino oligo added. 0.5 Molar tricine buffer, pH 9, was added to provide 25 millimolar final tricine concentration and left for 30 minutes on ice. 50 equivalents of sodium cyanoborohydride was then added  
15 and the pH reduced to 6.5 with acetic acid and reaction left for 60 minutes on ice. The resulting full length ribozyme was then purified for further analysis.

**Example 88: Amide Bond**

Referring again to Fig. 70 and 71, a 5' half of ribozyme was provided with a carboxyl group at its 2' position and was coupled with an amine containing  
20 3' half ribozyme. The provision of a coupling reagent resulted in a full-length ribozyme having an amide bond.

**Example 89: Disulfide Bond**

Referring to Fig. 70 and 71, 250 micrograms of RPI3881 and 250 micrograms of RPI3636 half ribozyme were separately deprotected with  
25 dithiothreitol overnight at 37°C. They were mixed together at 1:1 mole ratio in a 100 mM sodium phosphate buffer at pH 8 and 4M copper sulfate and 0.8 mM 1,10-phenanthroline (final concentrations) was added for two hours at room temperature (20-25°C) and the resulting mixture gel purified. The overall purification yield of full length ribozyme was 30%.

30 To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 1.8 KB region (containing site A) was synthesized by PCR using

primers that place the T7 RNA promoter upstream of the amplified sequence. Target RNA was transcribed, using T7 RNA polymerase, in a standard transcription buffer in the presence of [ $\alpha$ - $^{32}$ P]CTP. The reaction mixture was treated with 15 units of ribonuclease-free DNaseI, extracted with phenol followed chloroform:isoamyl alcohol (25:1), precipitated with isopropanol and washed with 70% ethanol. The dried pellet was resuspended in 20  $\mu$ l DEPC-treated water and stored at -20°C.

Unlabeled ribozyme (200 nM) and internally labeled 1.8 KB substrate RNA (<10 nM) were denatured and renatured separately in a standard cleavage buffer (containing 50 mM Tris-HCl pH 7.5 and 10 mM MgCl<sub>2</sub>) by heating to 90°C for 2 min. and slow cooling to 37°C for 10 min. The reaction was initiated by mixing the ribozyme and substrate mixtures and incubating at 37°C. Aliquots of 5  $\mu$ l were taken at regular time intervals, quenched by adding an equal volume of 2X formamide gel loading buffer and frozen on dry ice. The samples were resolved on 5% polyacrylamide sequencing gel and results were quantitatively analyzed by radioanalytic imaging of gels with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Few antiviral drug therapies are available that effectively inhibit established viral infections. Consequently, prophylactic immunization has become the method of choice for protection against viral pathogens. However, effective vaccines for divergent viruses such as those causing the common cold, and HIV, the etiologic agent of AIDS, may not be feasible. Consequently, new antiviral strategies are being developed for combating viral infections.

Gene therapy represents a potential alternative strategy, where antiviral genes are stably transferred into susceptible cells. Such gene therapy approaches have been termed "intracellular immunization" since cells expressing antiviral genes become immune to viral infection (Baltimore, 1988 *Nature* 335, 395-396). Numerous forms of antiviral genes have been developed, including protein-based antivirals such as transdominant inhibitory proteins (Malim et al., 1993 *J. Exp. Med.*, Bevec et al., 1992 *P.N.A.S. (USA)* 89, 9870-9874; Bahner et al., 1993 *J. Virol.* 67, 3199-3207) and viral-activated suicide genes (Ashom et al., 1990 *P.N.A.S.(USA)* 87, 8889-8893). Although



effective in tissue culture, protein-based antivirals have the potential to be immunogenic *in vivo*. It is therefore conceivable that treated cells expressing such foreign antiviral proteins will be eradicated by normal immune functions. Alternatives to protein based antivirals are RNA based molecules such as antisense RNAs, decoy RNAs, agonist RNAs, antagonist RNAs, therapeutic editing RNAs and ribozymes. RNA is not immunogenic; therefore, cells expressing such therapeutic RNAs are not susceptible to immune eradication.

Example 90: Design and construction of U6-S35 Chimera

A transcription unit, termed U6-S35, is designed that contains the characteristic intramolecular stem of a S35 motif (see Figure 76). As shown in Figure 77, 78 and 79 a desired RNA (e.g. ribozyme) can be inserted into the indicated region of U6-S35 chimera. This construct is under the control of a type 3 pol III promoter, such as a mammalian U6 small nuclear RNA (snRNA) promoter (see Fig. 75). U6-S35-HHI and U6-S35-HHII are non-limiting examples of the U6-S35 chimera.

As a non-limiting example, applicant has constructed a stable, active ribozyme RNA driven from a eukaryotic U6 promoter (Fig. 78). For stability, applicant incorporated a S35 motif as described in Fig. 76 and Fig. 77. A ribozyme sequence is inserted at the top of the stem, such that the ribozyme is separated from the S35 motif by an unstructured spacer sequence (Fig. 77, 78, 79). The spacer sequence can be customized for each desired RNA sequence. U6-S35 chimera is meant to be a non-limiting example and those skilled in the art will recognize that the structure disclosed in the figures 77, 78 and 79 can be driven by any of the known RNA polymerase promoters and are within the scope of this invention. All that is necessary is for the 5' region of a transcript to interact with its 3' region to form a stable intramolecular structure (S35 motif) and that the S35 motif is separated from the desired RNA by a stretch of unstructured spacer sequence. The spacer sequence appears to improve the effectiveness of the desired RNA.

By "unstructured" is meant lack of a secondary and tertiary structure such as lack of any stable base-paired structure within the sequence itself, and preferably with other sequences in the attached RNA.

By "spacer sequence" is meant any unstructured RNA sequence that separates the S35 domain from the desired RNA. The spacer sequence can be greater than or equal to one nucleotide.

*In vitro Catalytic Activity of U6-S35-Ribozyme Chimeras:*

5 U6-S35-HHI ribozyme RNA was synthesized using T7 RNA polymerase. HHI RNA was chemically synthesized using RNA phosphoramidite chemistry as described in Wincott et al., 1995 *Nucleic Acids Res.* The ribozyme RNAs were gel-purified and the purified ribozyme RNAs were heated to 55°C for 5 min. Target RNA used was ~650 nucleotide long. Internally-<sup>32</sup>P-labeled  
10 target RNA was prepared as described above. The target RNA was pre-heated to 37°C in 50 mM Tris.HCl, 10 mM MgCl<sub>2</sub> and then mixed at time zero with the ribozyme RNAs (to give 200 nM final concentration of ribozyme). At appropriate times an aliquot was removed and the reaction was stopped by  
15 dilution in 95% formamide. Samples were resolved on a denaturing urea-polyacrylamide gel and products were quantitated on a phosphorimager®.

As shown in Figure 80, the U6-S35-HHI ribozyme chimera cleaved its target RNA as efficiently as a chemically synthesized HHI ribozyme. In fact, it appears that the U6-S35-HHI ribozyme chimera may be more efficient than the synthetic ribozyme.

20 *Accumulation of U6-S35-ribozyme transcripts*

An Actinomycin D assay was used to measure accumulation of the transcript in mammalian cells. Cells were transfected overnight with plasmids encoding the appropriate transcription units (2µg DNA/well of 6 well plate) using calcium phosphate precipitation method (Maniatis et al., 1982 *Molecular*  
25 *Cloning* Cold Spring Harbor Laboratory Press, NY). After the overnight transfection, media was replaced and the cells were incubated an additional 24 hours. Cells were then incubated in media containing 5µg/ml Actinomycin D. At the times indicated, cells were lysed in guanidinium isothiocyanate, and total RNA was purified by phenol/chloroform extraction and isopropanol  
30 precipitation as described by Chomczynski and Sacchi, 1987 *Anal. Biochem.*, 162, 156. RNA was analyzed by northern blot analysis and the levels of

stromolysin, B7-1, B7-2, B7-3 and/or CD40 or other RNAs in a cell. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple ribozymes described in this invention, one may map nucleotide changes which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combinational therapies (e.g., multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other *in vitro* uses of ribozymes of this invention are well known in the art, and include detection of the presence of mRNAs associated with B7-1, B7-2, B7-3 and/or CD40 or other RNA related conditions. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

In a specific example, ribozymes which can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first ribozyme is used to identify wild-type RNA present in the sample and the second ribozyme will be used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA will be cleaved by both ribozymes to demonstrate the relative ribozyme efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates will also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus each analysis will require two ribozymes, two substrates and one unknown sample which will be combined into six reactions. The presence of cleavage products will be determined using an RNase protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and

- putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (*i.e.*, B7-1, B7-2, B7-3 and/or CD40) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a
- 5 qualitative comparison of RNA levels will be adequate and will decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios will be correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

Other embodiments are within the following claims.

**TABLE I****Characteristics of Ribozymes****Group I Introns**

Size: ~200 to >1000 nucleotides.

Requires a U in the target sequence immediately 5' of the cleavage site.

Binds 4-6 nucleotides at 5' side of cleavage site.

Over 75 known members of this class. Found in *Tetrahymena thermophila* rRNA, fungal mitochondria, chloroplasts, phage T4, blue-green algae, and others.

**RNaseP RNA (M1 RNA)**

Size: ~290 to 400 nucleotides.

RNA portion of a ribonucleoprotein enzyme. Cleaves tRNA precursors to form mature tRNA.

Roughly 10 known members of this group all are bacterial in origin.

**Hammerhead Ribozyme**

Size: ~13 to 40 nucleotides.

Requires the target sequence UH immediately 5' of the cleavage site.

Binds a variable number nucleotides on both sides of the cleavage site.

14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent (Figure 1)

**Hairpin Ribozyme**

Size: ~50 nucleotides.

Requires the target sequence GUC immediately 3' of the cleavage site.

Binds 4-6 nucleotides at 5' side of the cleavage site and a variable number to the 3' side of the cleavage site.

Only 3 known member of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, arabis mosaic virus and chicory yellow mottle virus) which uses RNA as the infectious agent (Figure 3).

**Hepatitis Delta Virus (HDV) Ribozyme**

Size: 50 - 60 nucleotides (at present).

Cleavage of target RNAs recently demonstrated.

Sequence requirements not fully determined.

Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required.

Only 1 known member of this class. Found in human HDV (Figure 4).

***Neurospora* VS RNA Ribozyme**

Size: ~144 nucleotides (at present)

Cleavage of target RNAs recently demonstrated.

Sequence requirements not fully determined.

Binding sites and structural requirements not fully determined. Only 1 known member of this class. Found in *Neurospora* VS RNA (Figure 5).

Table AII: Human Stromelysin Hammerhead Target Sequence

<u>nt</u> <u>Position</u>	<u>Sequence</u>	<u>SEQ. ID. NO.</u>
20	UAGAGCUAAGUAAAGCCAG	ID. NO. 01
126	ACACCAGCAUGAA	ID. NO. 02
147	AGAAAUUCUAGA	ID. NO. 03
171	ACCUCAAAAAGAUUGAAACAGU	ID. NO. 04
240	AAAUCCAGAAGUUC	ID. NO. 05
287	GACACUCUGGAGGUGAUGGCAAGCCCAGGUGU	ID. NO. 06
327	CUGAUGUUGGUCACUUCAGAAC	ID. NO. 07
357	GCAUCCCGAAGUGGAGGAAAACCCACCUUACAU	ID. NO. 08
402	AUUUAUACACCAGAUUUGCCAAAAGAU	ID. NO. 09
429	CUGUUGAUUCUGCUGUUGAGA	ID. NO. 10
455	CUGAAAGUCUGGAAGAGGUGA	ID. NO. 11
513	CUGAUUAUAUGA	ID. NO. 12
592	UGCCUAUGCCCC	ID. NO. 13
624	AUGCCCCACUUUGAUGAUGAUGAACAAUGGACA	ID. NO. 14
671	AUUUCUUGUUGCUGUCUAUG	ID. NO. 15
725	CACUCAGCCAACACUGA	ID. NO. 16
801	AAGAUGAUUAUAAUUGGCAUUCAGUCC	ID. NO. 17
827	CUCUAUGGACCUCCCCCUGACUCCCCU	ID. NO. 18
859	CCCCCUGGUACCCA	ID. NO. 19
916	UCCUGCUUUGUCCUUGAUGCUGUCAGCAC	ID. NO. 20
958	AAUCCUGAUUCUUUAAGA	ID. NO. 21
975	CAGGCACUUUUGGCGCAAUCCC	ID. NO. 22
1018	AUUGCAUUUGAUCUCUUCAUUUUGGCCAUC	ID. NO. 23
1070	GCAUAUGAAGUA	ID. NO. 24
1203	AAAUCCGAUGCAGCCAUUUCUGA	ID. NO. 25
1274	UUUGAUGAGAAGAGAAAUUCCAUUGGAGC	ID. NO. 26
1302	CAGGCUUCCCCAAGCAAUAAGCUGAAGAC	ID. NO. 27
1420	CCCAAUCCAAAG	ID. NO. 28
1485	AUGUAGAAGGCACAAUAUUGGGCACUUUAAA	ID. NO. 29
1623	UCUUGCCGGUCAUUUUUAUGUUAU	ID. NO. 30
1665	GCUGCUGCUUAGC	ID. NO. 31

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1733

CAACAGACAAGUGACUGUAUCU

ID. NO. 32

1769

CUUAUUUAAUA

ID. NO. 33



**Table AIII: Human Stromelysin HH Target Sequence**

nt. Position	Target Sequence	Seq. ID. NO.
10	GCAAGGCAUA GAGACAACAUAGAGC	ID. NO. 34
21	GCAUAGAGACAACAU GAGCUAAGUAAAGCC	ID. NO. 35
27	AGACAACAUAGAGCUA AGUAAAGCCAGUGGA	ID. NO. 36
31	AACAUAGAGCUAAGUA AAGCCAGUGGAAUUG	ID. NO. 37
53	GUGGAAUUGAAGAGUC UUCCAUCCUACUGU	ID. NO. 38
55	GGAAUUGAAGAGUCU CCAUCCUACUGUUG	ID. NO. 39
56	GAAUUGAAGAGUCUUC CAUCCUACUGUUGC	ID. NO. 40
61	GAAGAGUCUCCAUC CUACUGUUGCUGUGC	ID. NO. 41
64	GAGUCUCCAUCCUA CUGUUGCUGUGGUG	ID. NO. 42
69	UUCCAUCCUACUGU GCUGUGGUGGCAGU	ID. NO. 43
85	GCUGUGGUGGCAGUU UGCUCAGCCUAUCCA	ID. NO. 44
86	CUG UUGGCAGUUU GCUCAGCCUAUCCAU	ID. NO. 45
90	GCGUGGCAGUUUGCUC AGCCUAUCCAUUGGA	ID. NO. 46
96	CAGUUUGCUCAGCCUA UCCAUGGAUGGAGC	ID. NO. 47
98	GUUUGCUCAGCCUAC CAUUGGAUGGAGCUG	ID. NO. 48
102	GCUCAGCCUAUCCAU GGAUGGAGCUGCAAG	ID. NO. 49
142	CACCAGCAUGAACUU GUUCAGAAAUUCUA	ID. NO. 50
145	CAGCAUGAACCUUGUU CAGAAAUUCUAGAA	ID. NO. 51
146	AGCAUGAACCUUGUUC AGAAAUUCUAGAAA	ID. NO. 52
153	ACCUUGUUCAGAAUA UCUAGAAAACUACUA	ID. NO. 53
155	CUUGUUCAGAAAUUC UAGAAAACUACUACG	ID. NO. 54
157	UGUUCAGAAAUUCUA GAAACUACUACGAC	ID. NO. 55
165	AAUAUCUAGAAAACUA CUACGACCUCAAAAA	ID. NO. 56
168	AUCUAGAAAACUACUA CGACCUCAAAAAAGA	ID. NO. 57
175	AAACUACUACGACCUC AAAAAAGAUUGUGAAA	ID. NO. 58
195	AAGAUGUGAAACAGUU UGUAGGAGAAAGGA	ID. NO. 59
196	AGAUGUGAAACAGUU GUUAGGAGAAAGGAC	ID. NO. 60

199	UGUGAAACAGUUGUU AGGAGAAAGGACAGU	ID. NO. 61
200	GUGAAACAGUUGUUA GGAGAAAGGACAGUG	ID. NO. 62
218	AGAAAGGACAGUGGUC CUGUUGUAAAAAAAA	ID. NO. 63
223	GGACAGUGGUCCUGUU GUAAAAAAAAUCCGA	ID. NO. 64
226	CAGUGGUCCUGUUGUU AAAAAAAAAUCCGAGAA	ID. NO. 65
227	AGUGGUCCUGUUGUUA AAAAAAUCCGAGAAA	ID. NO. 66
235	UGUUGUAAAAAAAAUC CGAGAAUCCGAGAAG	ID. NO. 67
252	GAGAAUCCGAGAAGUU CCUUGGAUUGGAGGU	ID. NO. 68
253	AGAAUCCGAGAAGUUC CUUGGAUUGGAGGUG	ID. NO. 69
256	AUCCGAGAAGUCCUU GGAUUGGAGGUGAGC	ID. NO. 70
261	AGAAGUCCUUGGAUU GGAGGUGACGGGGAA	ID. NO. 71
285	CGGGAGCUGGACUC CGACACUCUGGAGGU	ID. NO. 72
293	CUGGACUCCGACACUC UGGAGGUGAUGGCA	ID. NO. 73
325	GCCCAGGUGUGGAGUU CCUGAUGUUGGUCAC	ID. NO. 74
326	CCCAGGUGUGGAGUUC CUGAUGUUGGUCACU	ID. NO. 75
334	UGGAGUCCUGAUGUU GGUCACUUCAGAAC	ID. NO. 76
338	GUCCUGAUGUUGGUC ACUUCAGAACCUUUC	ID. NO. 77
342	CUGAUGUUGGUCACUU CAGAACCUUCCUGG	ID. NO. 78
343	UGAUGUUGGUCACUUC AGAACCUUCCUGGC	ID. NO. 79
351	GUCACUUCAGAACCUU UCCUGGCAUCCCGAA	ID. NO. 80
352	UCACUUCAGAACCUU CCUGGCAUCCCGAAG	ID. NO. 81
353	CACUUCAGAACCUUUC CUGGCAUCCCGAAGU	ID. NO. 82
361	AACCUUCCUGGCAUC CCGAAGUGGAGGAAA	ID. NO. 83
385	GAGGAAACCCACCUU ACALACAGGAUUGUG	ID. NO. 84
386	AGGAAACCCACCUUA CALACAGGAUUGUGA	ID. NO. 85
390	AAACCCACCUUACALU CAGGAUUGUGAALUA	ID. NO. 86
397	CCUACALACAGGAUU GUGAALUALACACCA	ID. NO. 87
404	UACAGGAUUGUGAALU ALACACCGAUUUGC	ID. NO. 88
405	ACAGGAUUGUGAALUA UACACCGAUUUGCC	ID. NO. 89
407	AGGAUUGUGAALUALA CACCAGAUUUGCCAA	ID. NO. 90
416	AALUALACACCGAUU UGCCAAAAGAUCCUG	ID. NO. 91
417	AUALACACCGAUUU GCCAAAAGAUCCUGU	ID. NO. 92
433	GCCAAAAGAUCCUGUU GAUUCUGCUGUUGAG	ID. NO. 93
437	AAAGAUCCUGUUGAUU CUCUGUUGAGAAAG	ID. NO. 94
438	AAGAUCCUGUUGAUUC UGCUGUUGAGAAAGC	ID. NO. 95
445	UGUUGAUUCUGCUGU GAGAAAGCUCUGAAA	ID. NO. 96

455	GCUGUUGAGAAAGCUC UGAAAGUCUGGAAG	ID. NO. 97
463	GAAAGCUCUGAAAGUC UGGGAAGAGGUGACU	ID. NO. 98
479	UGGGAAGAGGUGACUC CACUCACAUUCUCCA	ID. NO. 99
484	AGAGGUGACUCCACUC ACAUUCUCCAGGCUG	ID. NO. 100
489	UGACUCCACUCACAUU CUCCAGGCUGUAUGA	ID. NO. 101
490	GACUCCACUCACAUUC UCCAGGCUGUAUGAA	ID. NO. 102
492	CUCCACUCACAUUCUC CAGGCUGUAUGAAGG	ID. NO. 103
501	CAUUCUCCAGGCUGUA UGAAGGAGAGGCUGA	ID. NO. 104
518	GAAGGAGAGGCUGAUA UAUGAUCUCUUUUG	ID. NO. 105
520	AGGAGAGGCUGAUAUA AUGAUCUCUUUUGCA	ID. NO. 106
526	GGCUGAUAUAUGAUC UCUUUUGCAGUAGA	ID. NO. 107
528	CUGAUAUAUGAUCUC UUUUGCAGUAGAGA	ID. NO. 108
530	GAUAUAUGAUCUCUU UUGCAGUAGAGAAC	ID. NO. 109
531	AUAUAUGAUCUCUUU UGCAGUAGAGAACA	ID. NO. 110
532	UAUAUGAUCUCUUUU GCAGUAGAGAACAU	ID. NO. 111
538	GAUCUCUUUUGCAGUU AGAGAACAUGGAGAC	ID. NO. 112
539	AUCUCUUUUGCAGUUA GAGAACAUGGAGACU	ID. NO. 113
555	GAGAACAUGGAGACUU UAACCCUUUGAUGG	ID. NO. 114
556	AGAACAUGGAGACUUU UAACCCUUUGAUGGA	ID. NO. 115
557	GAACAUGGAGACUUU UAACCCUUUGAUGGAC	ID. NO. 116
558	AACAUGGAGACUUUA CCCCUUUGAUGGACC	ID. NO. 117
563	GGAGACUUUUAACCCUU UGAUGGACCUGGAA	ID. NO. 118
564	GAGACUUUUAACCCUUU UGAUGGACCUGGAAA	ID. NO. 119
565	AGACUUUUAACCCUUU GAUGGACCUGGAAAU	ID. NO. 120
583	UGGACCUGGAAALGUU UGGGCCAUGCCUAAU	ID. NO. 121
584	GGACCUGGAAALGUUU UGGGCCAUGCCUAUG	ID. NO. 122
585	GACCUGGAAALGUUUU GGGCCAUGCCUAUGC	ID. NO. 123
597	UUUUGGGCCAUGCCUA UGGGCCUGGGCCAGG	ID. NO. 124
616	CCUGGGCCAGGGAUU AAUGGAGAUGCCAC	ID. NO. 125
617	CCUGGGCCAGGGAUUA AUGGAGAUGCCACU	ID. NO. 126
633	AUGGAGAUGCCACUU UGAUGAUGAUGAACA	ID. NO. 127
634	UGGAGAUGCCACUUU GAUGAUGAUGAACAA	ID. NO. 128
662	CA AUGGACAAAGGALUA CAACAGGGACCAAUU	ID. NO. 129
677	ACAACAGGGACCAAUU UAUUUCUGUUGCUG	ID. NO. 130
678	CAACAGGGACCAAUUU AUUUUCUGUUGCUGC	ID. NO. 131
679	AACAGGGACCAAUUUA UUUUCUGUUGCUGCU	ID. NO. 132

681-	CAGGGACCAAUUUAUU UCUUGUUGCUGCUCA	ID. NO. 133
682	AGGGACCAAUUUAUU CUUGUUGCUGCUCAU	ID. NO. 134
683	GGGACCAAUUUAUUC UUGUUGCUGCUCAUG	ID. NO. 135
685	GACCAAUUUAUUCUC GUUGCUGCUCAUGAA	ID. NO. 136
688	CAAUUAUUAUUCUGU GUUGCUGCUCAUGAAU	ID. NO. 137
695	UUUCUUGUUGCUGCUC AUGAAAUUGGCCACU	ID. NO. 138
703	UGCUGCUCAUGAAAUU GGCCACUCCUUGGGU	ID. NO. 139
711	AUGAAAUUGGCCACUC CCUGGGUCUCUUUCA	ID. NO. 140
719	GGCCACUCCUUGGGUC UCUUUCACUCAGCCA	ID. NO. 141
721	CCACUCCUUGGGUCUC UUUCACUCAGCCAAC	ID. NO. 142
723	ACUCCUUGGGUCUCUU UCACUCAGCCAACAC	ID. NO. 143
724	CUCCUUGGGUCUCUUU CACUCAGCCAACACU	ID. NO. 144
725	UCCUUGGGUCUCUUUC ACUCAGCCAACACUG	ID. NO. 145
729	UGGGUCUCUUUCACUC AGCCAACACUGAAGC	ID. NO. 146
746	GCCAACACUGAAGCUU UGAUGUACCCACUCU	ID. NO. 147
747	CCAACACUGAAGCUUU GAUGUACCCACUCUA	ID. NO. 148
753	CUGAAGCUUUGAUGUA CCCACUCUACUCUC	ID. NO. 149
760	UUUGAUGUACCCACUC UACUCUCACUCACA	ID. NO. 150
762	UGAUGUACCCACUCUA UCACUCACUCACAGA	ID. NO. 151
764	AUGUACCCACUCUAC ACUCACUCACAGACC	ID. NO. 152
768	ACCCACUCUACUCUC ACUCACAGACUUGAC	ID. NO. 153
772	ACUCUACUCACUCUC ACAGACUUGACUUGG	ID. NO. 154
785	CUCACAGACUUGACUC GGUUCCGCUUGUCUC	ID. NO. 155
789	CAGACUUGACUUGGUU CCGCUGUCUCAAGA	ID. NO. 156
790	AGACUUGACUUGGUUC CGCUGUCUCAAGAU	ID. NO. 157
798	CUUGGUUCCGCUUGUC UCAAGAUUAUUAUA	ID. NO. 158
800	CGGUUCCGCUUGUCUC AAGAUAUAUAUAUG	ID. NO. 159
809	CUGUCUCAAGAUAUA UAAAUUGGCAUUCAGU	ID. NO. 160
811	GUCUCAAGAUAUAUA AAUGGCAUUCAGUCC	ID. NO. 161
820	UGAUAUAUAUAUGGCAU CAGUCCUUAUGGA	ID. NO. 162
821	GUAUAUAUAUAUGGCAU AGUCCUUAUGGAC	ID. NO. 163
825	UAAAUUGGCAUUCAGUC CCUCUAUGGACCUCC	ID. NO. 164
829	UGGCAUUCAGUCCUUC UAUGGACCUCCCUCC	ID. NO. 165
831	GCAUUCAGUCCUUAUA UGGACCUCCCUCCGA	ID. NO. 166
839	UCCUUAUAUGGACCUUC CCCCUGACUCCCUCC	ID. NO. 167
849	GACCUCCCUCCUGACUC CCCCUGAGACCCCUCC	ID. NO. 168

868	UGAGACCCCCUUGGUA CCCAGGGAACUGUC	ID. NO. 169
883	ACCCAGGGAACUGUC CCUCCAGAACCUGG	ID. NO. 170
887	ACGGAACUGUCCUUC CAGAACUUGGAGGC	ID. NO. 171
917	CCAGCCAACUGUGAUC CUGCUUUGUCCUUG	ID. NO. 172
923	AACUGUGAUCUGCUU UGUCCUUGAUGCUG	ID. NO. 173
924	ACUGUGAUCUGCUU GUCCUUGAUGCUGU	ID. NO. 174
927	GUGAUCUGCUUUGUC CUUUGAUGCUGUCAG	ID. NO. 175
930	AUCCUGCUUUGUCCU UGAUGCUGUCAGCAC	ID. NO. 176
931	UCCUGCUUUGUCCU GAUGCUGUCAGCACU	ID. NO. 177
940	GUCCUUGAUGCUGUC AGCACUCUGAGGGGA	ID. NO. 178
947	GAUGCUGUCAGCACUC UGAGGGGAGAAUCC	ID. NO. 179
961	UCUGAGGGGAGAAUC CUGAUCUUUAAAGAC	ID. NO. 180
967	GGGAGAAUCCUGAUC UUUAAAGACAGGCAC	ID. NO. 181
969	GAGAAUCCUGAUCU UAAAGACAGGCACU	ID. NO. 182
970	AGAAUCCUGAUCUU AAAGACAGGCACUU	ID. NO. 183
971	GAAUCCUGAUCUUU AAGACAGGCACUUU	ID. NO. 184
984	UUAAGACAGGCACU UUGGCGCAAUCCU	ID. NO. 185
985	UAAAGACAGGCACUU UGGCGCAAUCCUC	ID. NO. 186
986	AAAGACAGGCACUUU GCGCAAUCCUCA	ID. NO. 187
996	ACUUUUGGCGCAAUC CCUCAGGAGCUUGA	ID. NO. 188
1000	UUGGCGCAAUCCUC AGGAGUUGAACCU	ID. NO. 189
1009	AUCCUCAGGAAGCU GAACUGAALUGCAU	ID. NO. 190
1020	AGCUUGAACUGAAU GCALUUGAUCUCUC	ID. NO. 191
1025	GAACUGAALUGCAU UGALUCUUCALUUU	ID. NO. 192
1026	AACUGAALUGCAUU GAUCUCUUCALUUUG	ID. NO. 193
1030	UGAALUGCALUUGAUC UCUUCALUUUGGCA	ID. NO. 194
1032	AALUGCALUUGAUC UCALUUUUGGCCAUC	ID. NO. 195
1034	UUGCALUUGAUCUCU CALUUUGGCCAUCUC	ID. NO. 196
1035	UGCALUUGAUCUCUC AUUUUGGCCAUCUCU	ID. NO. 197
1038	AUUUGAUCUCUUCALU UGGCCALUCUCUCC	ID. NO. 198
1039	UUUGAUCUCUUCALUU UGGCCALUCUCUCCU	ID. NO. 199
1040	UUGAUCUCUUCALUUU GGCCALUCUCUCCU	ID. NO. 200
1047	CUUCALUUUGGCCAUC UCUUCCUUCAGGCGU	ID. NO. 201
1049	UCALUUUUGGCCAUC UCCUUCAGGCGUGG	ID. NO. 202
1051	AUUUUGGCCAUCUCU CCUUCAGGCGUGGAU	ID. NO. 203
1052	UUUUGGCCAUCUCUC CUUCAGGCGUGGALG	ID. NO. 204

1055	UGGOCALUCUCUUCU CAGGOGUGGAUGOOG	ID. NO. 205
1056	GGOCALUCUCUUCUUC AGGOGUGGAUGOOGC	ID. NO. 206
1074	GOGUGGAUGOOGCALA UGAAGUACUAGCAA	ID. NO. 207
1081	UGOOGCALAUGAAGUU ACUAGCAAGGACCUC	ID. NO. 208
1082	GOOGCALAUGAAGUUA CUAGCAAGGACCUCG	ID. NO. 209
1085	GCALAUGAAGUUAUA GCAAGGACCUCGUU	ID. NO. 210
1096	UACUAGCAAGGACCUC GUUUUCALUUUUAAA	ID. NO. 211
1099	UAGCAAGGACCUCGUU UUCALUUUUAAAGGA	ID. NO. 212
1100	AGCAAGGACCUCGUU UCAUUUUUAAAGGAA	ID. NO. 213
1101	GCAAGGACCUCGUUU CALUUUUUAAAGGAAA	ID. NO. 214
1102	CAAGGACCUCGUUUC AUUUUUUAAAGGAAU	ID. NO. 215
1105	GGACCUCGUUUUCALU UUUAAAGGAAAUCAA	ID. NO. 216
1106	GACCUCGUUUUCALUU UUAAGGAAAUCAAU	ID. NO. 217
1107	ACCUCGUUUUCALUUU UAAAGGAAAUCAAU	ID. NO. 218
1108	CCUCGUUUUCALUUUU AAAGGAAAUCAAUC	ID. NO. 219
1109	CUCGUUUUCALUUUUA AAGGAAAUCAAUCU	ID. NO. 220
1118	AUUUUUAAAGGAAUUC AAUUCUGGGOCALCA	ID. NO. 221
1122	UUAAGGAAAUCAAUU CUGGGOCALCAGAGG	ID. NO. 222
1123	UAAAGGAAAUCAAUC UGGGOCALCAGAGGA	ID. NO. 223
1132	UCAAUUCUGGGOCALC AGAGGAAAUAGAGGUA	ID. NO. 224
1147	CAGAGGAAAUAGAGGUA CGAGCUGGALUACCCA	ID. NO. 225
1158	AGGUAAGAGCUGGALUA CCAAGAGGCAUCCA	ID. NO. 226
1171	ALUACCAAGAGGCCAUC CACACCCUAGGUUUC	ID. NO. 227
1180	AGGCAUCCACACCCUA GGUUCCCUCCAACC	ID. NO. 228
1184	AUCCACACCCUAGGUU UCCUCCCAACCGUGA	ID. NO. 229
1185	UCCACACCCUAGGUUU CCCUCCCAACCGUGAG	ID. NO. 230
1186	CCACACCCUAGGUUUC CCUCCCAACCGUGAGG	ID. NO. 231
1190	AACCUAGGUUCCCUUC CAACCGUGAGGAAAA	ID. NO. 232
1207	AAACCGUGAGGAAAAUC GAUGCAGCCAUUCU	ID. NO. 233
1219	AAUCGALGCAGCCAUU UCUGAUAAGGAAAAG	ID. NO. 234
1220	AUCGALGCAGCCAUUU CUGAUAAGGAAAAGA	ID. NO. 235
1221	UCGALGCAGCCAUUC UGAUAAGGAAAAGAA	ID. NO. 236
1226	GCAGCCAUUUCUGALUA AGGAAAAGAACAAAA	ID. NO. 237
1245	AAAAGAACAAAACALUA UUUUUUGUAGAGGA	ID. NO. 238
1247	AAGAACAAAACALUUA UUUUUUGUAGAGGACA	ID. NO. 239
1248	AGAACAAAACALUUAU CUUUUGUAGAGGACAA	ID. NO. 240

1249	GAACAAACAUUUUC UUUGUAGAGGACAAA	ID. NO. 241
1251	ACAAAACAUUUUCUU UGUAGAGGACAAUA	ID. NO. 242
1252	CAAAACAUUUUCUUU GUAGAGGACAAUAC	ID. NO. 243
1255	AACAUUUUCUUUGUA GAGGACAAAUACUGG	ID. NO. 244
1266	UUUGUAGAGGACAAUA CUGGAGAUUUGAUGA	ID. NO. 245
1275	ACAAAUACUGGAGAUU UGAUGAGAAGAGAAA	ID. NO. 246
1276	CAAUAUACUGGAGAUU GAUGAGAAGAGAAU	ID. NO. 247
1292	GAUGAGAAGAGAAAUU CCAUGGAGCCAGGCU	ID. NO. 248
1293	AUGAGAAGAGAAAUUC CAUGGAGCCAGGCUU	ID. NO. 249
1308	CCAUGGAGCCAGGCUU UCCCAAGCAAUAGC	ID. NO. 250
1309	CAUGGAGCCAGGCUUU CCCAAGCAAUAGCU	ID. NO. 251
1310	AUGGAGCCAGGCUUUC CCAAGCAAUAGCUG	ID. NO. 252
1321	CUUCCCAAGCAAUA GCUGAAGACUUUCCA	ID. NO. 253
1332	AAAUAGCUGAAGACUU UCCAGGGAUUGACUC	ID. NO. 254
1333	AUAGCUGAAGACUUU CCAGGGAUUGACUCA	ID. NO. 255
1334	AUAGCUGAAGACUUUC CAGGGAUUGACUCAA	ID. NO. 256
1342	AGACUUUCCAGGGAUU GACUCAAAGAUUGAU	ID. NO. 257
1347	UCCAGGGAUUGACUC AAAGAUUGAUGCUGU	ID. NO. 258
1354	GAUUGACUCAAGAUU GAUGCUGUUUUGAA	ID. NO. 259
1363	AAAGAUUGAUGCUGU UUUGAAGAUUUGGG	ID. NO. 260
1364	AAGAUUGAUGCUGUUU UUGAAGAUUUGGGU	ID. NO. 261
1365	AGAUUGAUGCUGUUU UGAAGAUUUGGGUU	ID. NO. 262
1366	GAUUGAUGCUGUUUU GAAGAUUUGGGUUC	ID. NO. 263
1374	CUGUUUUGAAGAUU UGGGUUCUUUUAUU	ID. NO. 264
1375	UGUUUUGAAGAUUU GGGUUCUUUUAUUC	ID. NO. 265
1380	UUGAAGAUUUGGGUU CUUUUAUUUCUUAC	ID. NO. 266
1381	UGAAGAUUUGGGUUC UUUUAUUUCUUACU	ID. NO. 267
1383	AAGAUAUUGGGUUCU UUAUUUCUUUACUGG	ID. NO. 268
1384	AGAUAUUGGGUUCUU UAUUUUCUUUACUGGA	ID. NO. 269
1385	GAAUUGGGUUCUUUU AUUUUCUUUACUGGAU	ID. NO. 270
1386	AUUUGGGUUCUUUUA UUUUUUACUGGALC	ID. NO. 271
1388	UUUGGGUUCUUUUAU UCUUUACUGGALCUU	ID. NO. 272
1389	UUGGGUUCUUUUAUU CUUUACUGGALCUUC	ID. NO. 273
1390	UGGGUUCUUUUAUUC UUUACUGGALCUUCA	ID. NO. 274
1392	GGUUCUUUUAUUCUU UACUGGALCUUCACA	ID. NO. 275
1393	GUUCUUUUAUUCUUU ACUGGALCUUCACAG	ID. NO. 276

1394	UUCUUUUUUUUUUUUA CUGGAUCUUCACAGU	ID. NO. 277
1401	AUUUCUUUACUGGALC UUCACAGUUGGAGUU	ID. NO. 278
1403	UUCUUUACUGGAUCUU CACAGUUGGAGUUUG	ID. NO. 279
1404	UCUUUACUGGAUCUUC ACAGUUGGAGUUUGA	ID. NO. 280
1410	CUGGAUCUUCACAGUU GGAGUUUGAOC AAA	ID. NO. 281
1416	CUUCACAGUUGGAGUU UGACCCAAALGCAA	ID. NO. 282
1417	UUCACAGUUGGAGUU GACCCAAALGCAAAG	ID. NO. 283
1448	AAAGUGACACACACUU UGAAGAGUACAGCU	ID. NO. 284
1449	AAGUGACACACACUU GAAGAGUACAGCUG	ID. NO. 285
1457	CACACUUUGAAGAGUA ACAGCUGGCUUAAU	ID. NO. 286
1468	GAGUACAGCUGGCUU AAUUGUGAAAGAGA	ID. NO. 287
1469	AGUACAGCUGGCUU AAUUGUGAAAGAGAU	ID. NO. 288
1472	AACAGCUGGCUUAAU GUUGAAAGAGAUAG	ID. NO. 289
1475	AGCUGGCUUAAUUGU GAAAGAGAUAGUAG	ID. NO. 290
1485	AAUUGUGAAAGAGAU UGUGAAGGCACAAU	ID. NO. 291
1489	UUGAAAGAGAUAGUA GAAGGCACAAUAGG	ID. NO. 292
1501	UGUGAAGGCACAAU UGGGCACUUUAAU	ID. NO. 293
1510	CACAAUAGGGCACUU UAAUAGAGCUAAU	ID. NO. 294
1511	ACAALUAGGGCACUU AAUAGAGCUAAUAA	ID. NO. 295
1512	CAALUAGGGCACUU AAUAGAGCUAAUAAU	ID. NO. 296
1522	ACUUUAAUAGAGCUA AUAUUCUUCACCUA	ID. NO. 297
1525	UUAAUAGAGCUAUA AUUCUUCACCUAAGU	ID. NO. 298
1528	AAUAGAGCUAAUAAU CUUCACCUAAGUCUC	ID. NO. 299
1529	AUGAGAGCUAAUAAU CUUCACCUAAGUCUCU	ID. NO. 300
1531	GAAGCUAALUUCUU CACCUAAGUCUCUGU	ID. NO. 301
1532	AAGCUAALUUCUUC ACCUAAGUCUCUGUG	ID. NO. 302
1537	AAUAAUUCUUCACCUA AGUCUCUGUGAAUUG	ID. NO. 303
1541	AUUCUUCACCUAAGUC UCUGUGAAUUGAAU	ID. NO. 304
1543	UCUUCACCUAAGUC UCUGUGAAUUGAAU	ID. NO. 305
1551	UAAGUCUCUGUGAAU GAAUUGUUGUUUC	ID. NO. 306
1559	UGUGAAUUGAAUUGU GUUUUCUUCUGGU	ID. NO. 307
1560	GUGAAUUGAAUUGU GUUUUCUUCUGGU	ID. NO. 308
1563	AAUUGAAUUGUUGU UUCUUCUGGUUGU	ID. NO. 309
1564	AUUAAUUGUUGUU UUCUUCUGGUUGU	ID. NO. 310
1565	UUGAAUUGUUGUUU CUUCUGGUUGGU	ID. NO. 311
1566	UGAAUUGUUGUUU CUUCUGGUUGGU	ID. NO. 312



1568.	AAAUUUUUUUUUUUC CUGGUGUGUGUGA	ID. NO. 313
1586	GCCUGUGUGUGACUC GAGUCACACUAAGG	ID. NO. 314
1591	UGCUGUGACUUGAGUC ACACUCAAGGGAACU	ID. NO. 315
1597	GACUUGAGUCACACUC AAGGGAACUUGAGCG	ID. NO. 316
1607	ACACUCAAGGGAACUU GAGGUGAAUUCUGA	ID. NO. 317
1618	AACUUGAGGUGAALUC UGUUUCUUGGCGUC	ID. NO. 318
1622	UGAGGUGAAUUCUGA UCUUGGCGUACUUU	ID. NO. 319
1624	AGGUGAAUUCUGUAC UUGGCGUACUUUUU	ID. NO. 320
1626	CGUGAAUUCUGUACUU GCGGUGUACUUUUU	ID. NO. 321
1633	CUGUACUUGGCGGUC AUUUUUUUGUUAUA	ID. NO. 322
1636	UAUCUUGGCGGUCAUU UUUUUGUUAUACAG	ID. NO. 323
1637	AUCUUGGCGGUCAUU UUAUGUUAUACAGG	ID. NO. 324
1638	UCUUGGCGGUCAUUU UAUUGUUAUACAGGG	ID. NO. 325
1639	CUUGGCGGUCAUUUU AUGUUAUACAGGGC	ID. NO. 326
1640	UUGGCGGUCAUUUUA UGUUAUACAGGGCA	ID. NO. 327
1644	CGGUCAUUUUUGUU AUUACAGGGCAUUA	ID. NO. 328
1645	GGUCAUUUUUGUUA UUAACAGGGCAUCAA	ID. NO. 329
1647	UCAUUUUUUGUUAU ACAGGGCAUCAAU	ID. NO. 330
1648	CAUUUUUUGUUAUA CAGGGCAUCAAUUG	ID. NO. 331
1657	GUUAUACAGGGCAU CAAUUGGCGUGGUC	ID. NO. 332
1658	UUAUACAGGGCAUUC AAUUGGCGUGGUCU	ID. NO. 333
1674	AAUUGGCGUGGUCU AGCUUGCAUUGUC	ID. NO. 334
1675	AAUUGGCGUGGCUUA GCUUGCAUUGUCA	ID. NO. 335
1679	GGCUGGUGUAGCUU GCAUUGUACAUUA	ID. NO. 336
1686	GCUUAGCUUGCAUUC GUCAUAGAGUGAU	ID. NO. 337
1689	UAGCUUGCAUUGUC ACAUAGAGUGAUUU	ID. NO. 338
1694	UGCAUUGUACAUUA GAGUACUUUUCCA	ID. NO. 339
1702	GUCAUAGAGUGAUC UUUCCAAGAGAAGG	ID. NO. 340
1704	CACAUAGAGUGAUUU UCCAAGAGAAGGGG	ID. NO. 341
1705	ACAUAGAGUGAUUUU CCAAGAGAAGGGGA	ID. NO. 342
1706	CAUAGAGUGAUUUUC CCAAGAGAAGGGGA	ID. NO. 343
1727	AGAAGGGGAAGCACUC GUGUGAACAGACAA	ID. NO. 344
1751	CAGACAAGUGACUGA UCUUGUAGACUUAU	ID. NO. 345
1753	GACAAGUGACUGAUC UGUGUAGACUUAUG	ID. NO. 346
1759	UGACUGUAUCUGUGA GACUAUUUGCUUAU	ID. NO. 347
1764	GUUUCUGUGUAGACUA UUUGCUUAUUUAUA	ID. NO. 348

1766	AUCUGUGUAGACUAAU UGCUAAUUUAAUAAA	ID. NO. 349
1767	UCUGUGUAGACUAAU GCUAAUUUAAUAAAG	ID. NO. 350
1771	UGUAGACUAAUUGCUU AAUUAUAAAGACGA	ID. NO. 351
1772	GUAGACUAAUUGCUA UUAUAAUAAAGACAU	ID. NO. 352
1774	AGACUAAUUGCUAAU UAUAUAAAGACGAUU	ID. NO. 353
1775	GACUAAUUGCUAAUU AAUAUAAAGACGAUUG	ID. NO. 354
1776	ACUAAUUGCUAAUUA AUAAAGACGAUUUGU	ID. NO. 355
1779	AUUUGCUAAUUUAUA AAGACGAUUUGUCAG	ID. NO. 356
1788	UUUAUAAAGACGAU UGUCAGUUGUUU	ID. NO. 357
1789	UUAUAUAAAGACGAU GUCAGUUGUUU	ID. NO. 358
1792	AUAUAAAGACGAUUGUC AGUUGUUU	ID. NO. 359

**Table AIV: Human Stromelysin HP Target Sequence**

nt. Position	Target Sequence	Seq. ID. NO.
66	CUACU GUU GCUGUGGUGGCAGU	ID. NO. 360
82	UGGCA GUU UGCUCAGCCUAUCCA	ID. NO. 361
192	AAACA GUU UGUUAGGAGAAAGGA	ID. NO. 362
430	AUGCU GUU GAIUCUGCUGUUGAG	ID. NO. 363
442	CUGCU GUU GAGAAAGCUCUGAAA	ID. NO. 364
775	UCACA GAC CUGACUUGGUUCCGC	ID. NO. 365
1360	AUGCU GUU UUUGAAGAAUUUGG	ID. NO. 366
1407	UCACA GUU GGAGUUUGACCCAAA	ID. NO. 367

**Table AV: Human HH Ribozyme Sequence**

nt. Position.	Ribozyme Sequence	Seq. ID.
10	GUUGUCUC CUGAAGAGCACGAAAGUGCGAA AUGCCUUG	ID.NO.375
21	UUAGCUC CUGAUGAGGCCGAAAGGCCGAA AUGUUGU	ID.NO.376
168	GAGGUCG CUGAUGAGGCCGAAAGGCCGAA AGUAGUU	ID.NO.377
616	CUCCAUU CUGAUGAGGCCGAAAGGCCGAA AUCCCUUG	ID.NO.378
617	UCUCCAU CUGAUGAGGCCGAAAGGCCGAA AAUCCCU	ID.NO.379
633	CAUCAUCA CUGAAGAGCACGAAAGUGCGAA AGUGGGCA	ID.NO.380
634	UCAUCAUC CUGAAGAGCACGAAAGUGCGAA AAGUGGGC	ID.NO.381
662	CCUGUUG CUGAUGAGGCCGAAAGGCCGAA AUCCUUU	ID.NO.382
711	ACCCAGG CUGAUGAGGCCGAAAGGCCGAA AGUGGCC	ID.NO.383
820	GGGACUG CUGAUGAGGCCGAAAGGCCGAA AUGCCAU	ID.NO.384
883	UCUGGAGG CUGAAGAGCACGAAAGUGCGAA ACAGGUUC	ID.NO.385
947	CCCCUCA CUGAUGAGGCCGAAAGGCCGAA AGUGCUG	ID.NO.386
996	CCUGAGG CUGAUGAGGCCGAAAGGCCGAA AUUUGCG	ID.NO.387
1123	UGGCCCA CUGAUGAGGCCGAAAGGCCGAA AAUUGAU	ID.NO.388
1132	UUUCCUCU CUGAUGAGCACGAAAGUGCGAA AUGGCCCA	ID.NO.389
1221	CCUUAUCA CUGAAGAGCACGAAAGUGCGAA AAAUGGCU	ID.NO.390
1266	UCUCCAG CUGAUGAGGCCGAAAGGCCGAA AUUUGUC	ID.NO.391
1275	UCUCAUCA CUGAAGAGCACGAAAGUGCGAA AUCUCCAG	ID.NO.392
1334	AUCCCUUG CUGAUGAGGCCGAAAGGCCGAA AAAGUCU	ID.NO.393
1354	CAGCAUC CUGAUGAGGCCGAAAGGCCGAA AUCUUUG	ID.NO.394
1363	UCUUCAAA CUGAUGAGCACGAAAGUGCGAA ACAGCAUC	ID.NO.395
1410	AAACUCC CUGAUGAGGCCGAAAGGCCGAA ACUGUGA	ID.NO.396

Table AVI: Rabbit Stromelysin HH Ribozyme Target Sequence

nt. Position	Target Sequence	nt. Position	Target Sequence
18	CAAGGCAU C AAGACAGC	345	CCUGAUGU U GGUCACUU
29	GACAGCAU A GAGCUGAG	349	AUGUUGGU C ACUUCAGU
39	AGCUGAGU A AAGCCAAU	353	UGGUCACU U CAGUACCU
61	UGAAAACU C UUCCAACC	354	GGUCACUU C AGUACCTU
63	AAAACUCU U CCAACCCU	358	ACUUCAGU A CCUUCCTU
64	AAACUCUU C CAACCCUG	362	CAGUACCU U CCCUGGCA
75	ACCCUGCU A CUGCUGUG	363	AGUACCTU C CCUGGCAC
93	GUGGCGCU U UGCUCAGC	391	CAAAAACU C ACCUAACTU
94	UGGCGCTU U GTCUCAGC	396	ACUCACCU A ACUUCACG
98	GCTUUGCU C AGCCTAUC	400	ACCUAACTU U ACAGGAUU
104	CUCAGCCU A UCCACUGG	401	CCUAACTU A CAGGAUUG
106	CAGCCUAA C CACUGGAU	408	UACAGGAU U GUGAAUUA
122	UGGAGCCU C AAGGGAUG	415	UUGUGAAU U ACACACCG
153	AUGGACCU U CUUGAGCA	416	UGUGAAUU A CACACCGG
154	UGGACCTU C UUCAGCAA	427	CACCGGAU C UGCCAAGA
156	GACCTUCU U CAGCAAUA	444	GAUGCUGU U GAUGCUGC
157	ACCTUCUU C AGCAAUAU	456	GCTGCCAU U GAGAAAGC
164	UCAGCAAU A UCTGGAAA	466	AGAAAGCU C UGAAGGUC
166	AGCAAUAU C UGGAAAAC	474	CUGAAGGU C UGGGAGGA
176	GGAAAACU A CUACAACC	490	AGGUGACU C CACUCACG
179	AAACUACU A CAACCUUG	495	ACUCCACU C ACGUUCUC
186	UACAACCU U GAAAAAGA	500	ACUCACGU U CUCCAGGA
206	GAAACAGU U UGUUAAAA	501	CUCACGUU C UCCAGGAA
207	AAACAGUU U GUUAAAAG	503	CACGUUCU C CAGGAAGU
210	CAGUUUGU U AAAAGAAA	512	CAGGAAGU A UGAAGGAG
211	AGUUUGUU A AAAGAAAG	531	GCTUGACU A AUGAUCUC
226	AGGACAGU A GUCCUGUU	537	AUAAUGAU C UCUUUUGG
229	ACAGUAGU C CUGUUGUU	539	AAUGAUCU C UUUUGGAG
234	AGUCCUGU U GUUAAAAA	541	UGAUCUCU U UUGGAGUC
237	CCUGUUGU U AAAAAAAU	542	GAUCUCUU U UGGAGUCC
238	CUGUUGUU A AAAAAAUC	543	AUCUCUUU U GGAGUCCG
246	AAAAAAAU C CAAGAAAU	549	UUUGGAGU C CGAGAACA
263	GCAGAAGU U CCUUGGCU	565	AUGGAGAU U UUAUCCCU
264	CAGAAGUU C CUUGGCUU	566	UGGAGAUU U UAUUCCUU
267	AAGUUCUU U GGCUUGGA	567	GGAGAUUU U AUUCCUUU
272	CCUUGGCU U GGAGGUGA	568	GAGAUUUU A UUCCUUUU
296	GCTUGGACU C CAACACCC	570	GAUUUUUU U CCUUUUGA
315	GAGGUGAU A CGCAAGCC	571	AUUUUUUU C CUUUUGAU
336	UGUGGCGU U CCUGAUGU	574	UUUAUCCU U UUGAUGGA
337	GUGGCGUU C CUGAUGUU	575	UAUUCCUU U UGAUGGAC

576	AUUCUUU U GAUGGACC	905	UCCAGGAU C UGGGACCC
594	GGAAAGU U UUGGCUA	918	ACCCAGU C AUGUGUGA
595	GAAAGUU U UGGCUAU	928	UGUGUGAU C CAGAUUCG
596	AAAGUUU U GGCUCUAG	934	AUCCAGAU C UGUCCUUC
601	UUUUGGU C AUGCUAU	938	AGAUCUGU C CUUCGAUG
607	CUCAUGU U AUGCACC	941	UCUGUCCU U CGAUGCAA
608	UCAUGGU A UGCACUG	942	CUGUCCU C GAUGCAAU
627	CCAGGAU U AAUGGAGA	951	GAUGCAAU C AGCACUCU
628	CAGGAUU A AUGGAGAU	958	UCAGCAU C UGAGGGGA
644	UGCCCAU U UGAUGAU	972	GGAGAAU U CUGUUCU
645	GCCCAU U GAUGAUGA	973	GAGAAU C UGUUCUU
673	CAAAGGAU A CAACAGGA	977	AAUUCUGU U CUUUAAG
688	GAACCAU U UAUCCU	978	AUUCUGU C UUAAGA
689	AACCAU U AUCCUUG	980	UCUGUUCU U UAAAGACA
690	ACCAUUU A UUCUUGU	981	CUGUUCU U AAAGACAG
692	CAUUUAU U CCUUGUG	982	UGUUCUU A AAGACAGG
693	AAUUUAU C CUUGUGC	992	AGACAGGU A UUCUGGC
696	UUAUCCU U GUUGUGC	994	ACAGGUU U UCUGGCGC
699	UUCUUGU U GUGCUA	995	CAGGUU U CUGGCGCA
706	UUGUGU C AUGAGCU	996	AGGUUU C UGGCGCAA
714	CAUGAGU U GGCCACU	1007	GCGCAAGU C CCUCAGGA
722	UGGCCAU C GUGGUC	1011	AAGUCCU C AGGAUUCU
730	CCUGGGU C UGUUAC	1017	CUCAGGAU U CUCGAACC
734	GGUGUGU U UCACUGG	1018	UCAGGAU C UCGAACCU
735	GGUGUGU U CACUGGC	1020	AGGAUUCU C GAACCUA
736	GUGUGUU C ACUGGCC	1031	ACCUGAGU U UCAUUGA
741	GUUACAU C GGCCAACC	1032	CCUGAGU U CAUUGAU
741	GUUACAU C GGCCAACC	1033	CUGAGUU C AUUGAUC
771	UACCCAGU C UACAACG	1036	AGUUCAU U UGAUCUCU
773	CCCAGUCU A CAACGCCU	1037	GUUCAU U GAUCUCU
782	CAACGCCU U CACAGACC	1041	CAUUGAU C UCUCAU
783	AACGCCU C ACAGACCU	1043	UUUGAU C UUCAUUCU
800	GGCCCGU U CCGCCUU	1045	UGAUUCU U CAUUCUGG
801	GCCCGGU C CGCCUUC	1046	GAUCUCU C AUUCUGGC
807	UUCCGCU U UCUCAGA	1049	CUCUCAU U CUGGCCAU
808	UCCGCCU U UCUCAGA	1050	UCUCAU C UGGCCAUC
809	CCGCCUU C UCAAGAUG	1058	CUGGCCAU C UCUCCU
811	GCCUUUCU C AAGAUGAU	1060	GGCAUCU C UUCUUA
831	GAUGGCAU C CAAUCCU	1062	CCAUCU U CUCUAGC
836	CAUCCAU C CCUCUAG	1063	CAUCUCU C CUUCAGCA
840	CAAUCCU C UAGGACC	1066	CUCUCCU U CAGCAGUG
842	AUCCUCU A UGGACCGG	1067	UCUCCU C AGCAGUGG
860	CCUUGCU C UCUGAUA	1085	UGUGCAU A UGAAGUA
862	CUGCCUCU C CUGAUAC	1092	UAUGAGU U AUUAGCAG
868	CUCCUGAU A ACUCUGGA	1093	AUGAAGU A UUAGCAGG
872	UGAUACU C UGGAGUGC	1095	GAAGUAU U AGCAGGGA
883	GAGUGCCU A UGGAACCU	1096	AAGUUAU A GCAGGGAU
894	GAACCGU C CCUCAGG	1105	GCAGGGAU A CUGUUUC
898	CUGUCCU C CAGGAUCU	1110	GAUUCU U UUCAUUU

1111	AUACUGUU U UCAUUUUU	1374	GAUGCUGU U UUUGAAGC
1112	UACUGUUU U CAUUUUUA	1375	AUGCUGUU U UUGAAGCA
1113	ACUGUUUU C AUUUUUAA	1376	UGCUGUUU U UGAAGCAU
1116	GUUUUCAU U UUUAAAGG	1377	GCUGUUUU U GAAGCAUU
1117	UUUUCAUU U UUAAGGA	1385	UGAAGCAU U UGGGUUUU
1118	UUUCAUUU U UAAAGGAA	1386	GAAGCAUU U GGGUUUUU
1119	UUCAUUUU U AAAGGAAC	1391	AUUUGGGU U UUUCUAUU
1120	UCAUUUUU A AAGGAACU	1392	UUUGGGUU U UUCUAUUU
1129	AAGGAACU C AGUUCUGG	1393	UUGGGUUU U UCUAUUUC
1133	AACUCAGU U CUGGGCCA	1394	UGGGUUUU U CUAUUUCU
1134	ACUCAGUU C UGGGCCAU	1395	GGGUUUUU C UAUUUCUU
1143	UGGGCCAU U AGAGGAAA	1397	GUUUUUUU A UUUCUACA
1144	GGGCCAUU A GAGGAAAU	1399	UUUUCUAU U UCUUCAGU
1158	AAUGAGGU A CAAGCUGG	1400	UUUCUAUU U CUUCAGUG
1168	AAGCUGGU U ACCCAAGA	1401	UUCUAUUU C UUCAGUGG
1169	AGCUGGUU A CCCAAGAA	1403	CUAUUUUU U CAGUGGAU
1182	AGAAGCAU C CACACCCU	1404	UAUUUCUU C AGUGGAUC
1195	CCCUGGGU U UCCCUUCA	1412	CAGUGGAU C UUCACAGU
1196	CCUGGGUU U CCCUUCAA	1414	GUGGAUCU U CACAGUCG
1197	CUGGGUUU C CCUUCAAC	1415	UGGAUCUU C ACAGUCGG
1201	GUUUCUUU U CAACCAUA	1421	UUCACAGU C GGAGUUUG
1202	UUUCCUUU C AACCAUAA	1427	GUCCGAGU U UGACCCAA
1209	UCAACCAU A AGAAAAAU	1428	UCGGAGUU U GACCCAAA
1218	AGAAAAAU U GAUGCUGC	1458	ACACAUGU U UUGAAGAG
1230	GCUGCCAU U UCUGAUAA	1459	CACAUGUU U UGAAGAGC
1231	CUGCCAUU U CUGAUUAG	1460	ACAUGUUU U GAAGAGCA
1232	UGCCAUUU C UGAUAAGG	1478	CAGCUGGU U UCAGUGUU
1237	UUUCUGAU A AGGAAAGG	1479	AGCUGGUU U CAGUGUUA
1256	GAAAACAU A CUUCUUUG	1480	GCUGGUUU C AGUGUUAG
1259	AACAUACU U CUUUGUGG	1486	UUCAGUGU U AGGAGGGG
1260	ACAUACUU C UUUGUGGA	1487	UCAGUGUU A GGAGGGGU
1262	AUACUUCU U UGUGGAAG	1498	AGGGGUGU A UAGAGGGC
1263	UACUUCUU U GUGGAAGA	1500	GGGUGUUA A GAAGGCAC
1277	AGACAAAU A CUGGAGGU	1519	AUGAAUGU U UUAUAUGA
1286	CUGGAGGU U UGAUGAGA	1520	UGAAUGUU U UAAUGAA
1287	UGGAGGUU U GAUGAGAA	1521	GAAUGUUU U AAAUGAAC
1304	GAGACAGU C CCUGGAGC	1522	AAUGUUUU A AAUGAACC
1319	GCCAGGCU U UCCAGAC	1532	AUGAACCU A AUUGUACA
1320	CCAGGCUU U CCCAGACA	1535	AACCUAAU U GUUCAACA
1321	CAGGCUUU C CCAGACAU	1538	CUAAUUGU U CAACACUU
1330	CCAGACAU A UAGCAGAA	1539	UAUUUGUU C AACACUUA
1332	AGACAUUU A GCAGAAGA	1546	UCAACACU U AGGACUUU
1343	AGAAGACU U UCCAGGAA	1547	CAACACUU A GGACUUUG
1344	GAAGACUU U CCAGGAAU	1553	UUAGGACU U UGUGAGUU
1345	AAGACUUU C CAGGAAUU	1554	UAGGACUU U GUGAGUUG
1353	CCAGGAAU U AAUCCAAA	1561	UUUGAGAU U GAAGUGGC
1354	CAGGAAUU A AUCCAAAG	1571	AAGUGGCU C AUUUUCUC
1357	GAAUUAAU C CAAAGAUC	1574	UGGCUCAU U UUCUCCUG
1365	CCAAAGAU C GAUGCUGU	1575	GGCUCAUU U UCUCUCCG

1576 GCUCAUUU U CUCCUGCA  
1577 CUCAUUUU C UCCUGCAU  
1579 CAUUUUUC C CUGCAUUA  
1586 UCCUGCAU A UGCUGUGA  
1602 AUGGGAAU C UCGAGCAU  
1604 GGGAAUCU C GAGCAUGA  
1620 AACUGUGU A UCUAACUG  
1622 CUGUGUAU C UAACUGGA  
1624 GUGUAUCU A ACUGGACU  
1633 ACUGGACU U UGCACAUC  
1634 CUGGACUU U GCACAUUG  
1641 UUGCACAU C GUUACGGG  
1644 CACAUCGU U ACGGGUGU  
1645 ACAUCGUU A CGGGUGUU  
1653 ACGGGUGU U CAAACAGG  
1654 CGGGUGUU C AAACAGGC  
1670 CUGCUGCU U AGCUUGCA  
1671 UGCUGCUU A GCUUGCAC  
1675 GCUUAGCU U GCACUUGA  
1681 CUUGCACU U GAUCACAU  
1685 CACUUGAU C ACAUGGAA  
1701 AGGGAGCU U CCAAGAGA  
1702 GGGAGCUU C CACGAGAC  
1720 GGGGAAGU A CUCAUGUG  
1723 GAAGUACU C AUGUGUGA  
1744 CGAGUGAU U GUGUCUAU  
1749 GAUUAUUU C UAUGUGGA  
1751 UUAUUUUU A UGUGGAUU  
1759 AUGUGGAU U AUUUGCCC  
1760 UGUGGAUU A UUUGCCCA  
1762 UGGAUUUA U UGCCCAUU  
1763 GGAUUUAU U GCCCAUUA  
1770 UUGCCCAU U AUUUAAUA  
1771 UGCCCAUU A UUUAUAUA  
1773 CCCAUUAU U UAAUAAGG  
1774 CCAUUAUU U AAUAAAGA  
1775 CAUUAUUU A AUAAAGAG  
1778 UAUUUAUU A AAGAGGAU  
1787 AAGAGGAU U UGUCAAUU



Table AVII: Rabbit Stromelysin HH Ribozyme Sequenc

nt. Position	Ribozyme Sequence
18	GCUGUCUU CUGAUGAGGCCGAAAGGCCGAA AUGCCUUG
29	CUCAGCUC CUGAUGAGGCCGAAAGGCCGAA AUGCUGUC
39	AUUGGCUU CUGAUGAGGCCGAAAGGCCGAA ACUCAGCU
61	GGUUGGAA CUGAUGAGGCCGAAAGGCCGAA AGUUUUCA
63	AGGGUUGG CUGAUGAGGCCGAAAGGCCGAA AGAGUUUU
64	CAGGGUUG CUGAUGAGGCCGAAAGGCCGAA AAGAGUUU
75	CACAGCAG CUGAUGAGGCCGAAAGGCCGAA AGCAGGGU
93	GCUGAGCA CUGAUGAGGCCGAAAGGCCGAA AGCGCCAC
94	GGCUGAGC CUGAUGAGGCCGAAAGGCCGAA AAGCGCCA
98	GAUAGGCU CUGAUGAGGCCGAAAGGCCGAA AGCAAAGC
104	CCAGUGGA CUGAUGAGGCCGAAAGGCCGAA AGGCUGAG
106	AUCCAGUG CUGAUGAGGCCGAAAGGCCGAA AUAGGCUG
122	CAUCCCUU CUGAUGAGGCCGAAAGGCCGAA AGGCUCCA
153	UGCUGAAG CUGAUGAGGCCGAAAGGCCGAA AGGUCCAU
154	UUGCUGAA CUGAUGAGGCCGAAAGGCCGAA AAGGUCCA
156	UAUUGCUG CUGAUGAGGCCGAAAGGCCGAA AGAAGGUC
157	AUAUUGCU CUGAUGAGGCCGAAAGGCCGAA AAGAAGGU
164	UUUCCAGA CUGAUGAGGCCGAAAGGCCGAA AUUGCUGA
166	GUUUUCCA CUGAUGAGGCCGAAAGGCCGAA AUAUUGCU
176	GGUUGUAG CUGAUGAGGCCGAAAGGCCGAA AGUUUUCC
179	CAAGGUUG CUGAUGAGGCCGAAAGGCCGAA AGUAGUUU
186	UCUUUUUC CUGAUGAGGCCGAAAGGCCGAA AGGUUGUA
206	UUUUAACA CUGAUGAGGCCGAAAGGCCGAA ACUGUUUC
207	CUUUUAAC CUGAUGAGGCCGAAAGGCCGAA AACUGUUU
210	UUUCUUUU CUGAUGAGGCCGAAAGGCCGAA ACAAACTU
211	CUUUCUUU CUGAUGAGGCCGAAAGGCCGAA AACAAACU
226	AACAGGAC CUGAUGAGGCCGAAAGGCCGAA ACUGUCCU
229	AACAACAG CUGAUGAGGCCGAAAGGCCGAA ACUACUGU
234	UUUUUAAC CUGAUGAGGCCGAAAGGCCGAA ACAGGACT
237	AUUUUUUU CUGAUGAGGCCGAAAGGCCGAA ACAACAGG
238	GAUUUUUU CUGAUGAGGCCGAAAGGCCGAA AACAACAG
246	AUUUCUUG CUGAUGAGGCCGAAAGGCCGAA AUUUUUUU
263	AGCCAAGG CUGAUGAGGCCGAAAGGCCGAA ACUUCUGC
264	AAGCCAAG CUGAUGAGGCCGAAAGGCCGAA AACUUCUG
267	UCCAAGCC CUGAUGAGGCCGAAAGGCCGAA AGGAACTU
272	UCACCUCC CUGAUGAGGCCGAAAGGCCGAA AGCCAAGG
296	GGGUGUUG CUGAUGAGGCCGAAAGGCCGAA AGUCCAGC
315	GGCUUGCG CUGAUGAGGCCGAAAGGCCGAA AUCACCTC
336	ACAUCAGG CUGAUGAGGCCGAAAGGCCGAA ACGCCACA
337	AACAUCAG CUGAUGAGGCCGAAAGGCCGAA AACGCCAC
345	AAGUGACC CUGAUGAGGCCGAAAGGCCGAA ACAUCAGG

349 ACUGAAGU CUGAUGAGGCCGAAAGGCCGAA ACCAACAU  
 353 AGGUACTUG CUGAUGAGGCCGAAAGGCCGAA AGUGACCA  
 354 AAGGUACU CUGAUGAGGCCGAAAGGCCGAA AAGUGACC  
 358 AGGGAAGG CUGAUGAGGCCGAAAGGCCGAA ACUGAAGU  
 362 UGCCAGGG CUGAUGAGGCCGAAAGGCCGAA AGGUACTUG  
 363 GUGCCAGG CUGAUGAGGCCGAAAGGCCGAA AAGGUACU  
 391 AGUUAGGU CUGAUGAGGCCGAAAGGCCGAA AGUUUUUG  
 396 CUGUAAGU CUGAUGAGGCCGAAAGGCCGAA AGGUGAGU  
 400 AAUCCUGU CUGAUGAGGCCGAAAGGCCGAA AGUUAGGU  
 401 CAAUCCUG CUGAUGAGGCCGAAAGGCCGAA AAGUUAGG  
 408 UAAUUCAC CUGAUGAGGCCGAAAGGCCGAA AUCCUGUA  
 415 CGGUGUGU CUGAUGAGGCCGAAAGGCCGAA AUUCACAA  
 416 CCGGUGUG CUGAUGAGGCCGAAAGGCCGAA AAUUCACA  
 427 UCUUGGCA CUGAUGAGGCCGAAAGGCCGAA AUCCGGUG  
 444 GCAGCAUC CUGAUGAGGCCGAAAGGCCGAA ACAGCAUC  
 456 GCUUUCUC CUGAUGAGGCCGAAAGGCCGAA AUGGCAGC  
 466 GACCUUCA CUGAUGAGGCCGAAAGGCCGAA AGCUUUCU  
 474 UCCUCCCA CUGAUGAGGCCGAAAGGCCGAA ACCUUCAG  
 490 CGUGAGUG CUGAUGAGGCCGAAAGGCCGAA AGUCACCU  
 495 GAGAACGU CUGAUGAGGCCGAAAGGCCGAA AGUGGAGU  
 500 UCCUGGAG CUGAUGAGGCCGAAAGGCCGAA ACGUGAGU  
 501 UUCUUGGA CUGAUGAGGCCGAAAGGCCGAA AACGUGAG  
 503 ACUUCCTUG CUGAUGAGGCCGAAAGGCCGAA AGAACGUG  
 512 CUCCUUCA CUGAUGAGGCCGAAAGGCCGAA ACTUCCUG  
 531 GAGAUCAU CUGAUGAGGCCGAAAGGCCGAA AUGUCAGC  
 537 CCAAAAGA CUGAUGAGGCCGAAAGGCCGAA AUCAUUUA  
 539 CUCCAAAA CUC AGGCCGAAAGGCCGAA AGAUCAUU  
 541 GACUCCAA CUC AUGAGGCCGAAAGGCCGAA AGAGAUCA  
 542 GGACUCCA CUGAUGAGGCCGAAAGGCCGAA AAGAGAUU  
 543 CGGACUCC CUGAUGAGGCCGAAAGGCCGAA AAAGGAU  
 549 UGUUCUCG CUGAUGAGGCCGAAAGGCCGAA ACUCCAAA  
 565 AGGAUAUA CUGAUGAGGCCGAAAGGCCGAA AUCUCCAU  
 566 AAGGAUAU CUGAUGAGGCCGAAAGGCCGAA AAUUCUCA  
 567 AAAGGAU CUGAUGAGGCCGAAAGGCCGAA AAAUCUCC  
 568 AAAAGGAA CUGAUGAGGCCGAAAGGCCGAA AAAAUCUC  
 570 UCAAAAGG CUGAUGAGGCCGAAAGGCCGAA AUA AAAUC  
 571 AUCAAAAG CUGAUGAGGCCGAAAGGCCGAA AAUAAAUA  
 574 UCCAUCAA CUGAUGAGGCCGAAAGGCCGAA AGGAUAUA  
 575 GUCCAUCA CUGAUGAGGCCGAAAGGCCGAA AAGGAUAU  
 576 GGUCCAUC CUGAUGAGGCCGAAAGGCCGAA AAAGGAU  
 594 UGAGCCAA CUGAUGAGGCCGAAAGGCCGAA ACAUUUCC  
 595 AUGAGCCA CUGAUGAGGCCGAAAGGCCGAA AACAUUUC  
 596 CAUGAGCC CUGAUGAGGCCGAAAGGCCGAA AAACAUAU  
 601 AUAAGCAU CUGAUGAGGCCGAAAGGCCGAA AGCCAAAA  
 607 AGGUGCAU CUGAUGAGGCCGAAAGGCCGAA AGCAUGAG  
 608 CAGGUGCA CUGAUGAGGCCGAAAGGCCGAA AAGCAUGA  
 627 UCUCCAUU CUGAUGAGGCCGAAAGGCCGAA AUUCCUGG  
 628 AUCUCCAU CUGAUGAGGCCGAAAGGCCGAA AAUUCCTUG  
 644 CAUCAUCA CUGAUGAGGCCGAAAGGCCGAA AGUGGCA

645	UCAUCAUC	CUGAUGAGGCCGAAAGGCCGAA	AAGUGGGC
673	UCCUGUUG	CUGAUGAGGCCGAAAGGCCGAA	AUCCUUUG
688	AAGGAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUUGGUUC
689	CAAGGAU	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGUU
690	ACAAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGGU
692	CAACAAGG	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUUG
693	GCAACAAG	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAUU
696	GCAGCAAC	CUGAUGAGGCCGAAAGGCCGAA	AGGAAUAA
699	UGAGCAGC	CUGAUGAGGCCGAAAGGCCGAA	ACAAGGAA
706	AAGCUCAU	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCAA
714	GAGUGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGCUCAUG
722	GACCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGUGGCCA
730	GUGAAACA	CUGAUGAGGCCGAAAGGCCGAA	ACCCAGGG
734	CCGAGUGA	CUGAUGAGGCCGAAAGGCCGAA	ACAGACCC
735	GCCGAGUG	CUGAUGAGGCCGAAAGGCCGAA	AACAGACC
736	GGCCGAGU	CUGAUGAGGCCGAAAGGCCGAA	AAACAGAC
740	GGUUGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGUGAAAC
764	AGACUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAUCAGC
771	GCGUUGUA	CUGAUGAGGCCGAAAGGCCGAA	ACUGGGUA
773	AGGCGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGACUGGG
782	GGUCUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGGCGUUG
783	AGGUCUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGGCGUU
800	AAAGGCGG	CUGAUGAGGCCGAAAGGCCGAA	ACCGGGCC
801	GAAAGGCG	CUGAUGAGGCCGAAAGGCCGAA	AACCGGCC
807	UCUUGAGA	CUGAUGAGGCCGAAAGGCCGAA	AGGCGGAA
808	AUCUUGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCGGA
809	CAUCUUGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGGCGG
811	AUCAUCUU	CUGAUGAGGCCGAAAGGCCGAA	AGAAAGGC
831	AGGGAUUG	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAUC
836	CAUAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AUUGGAUG
840	GGUCCAUA	CUGAUGAGGCCGAAAGGCCGAA	AGGGAUUG
842	CCGGUCCA	CUGAUGAGGCCGAAAGGCCGAA	AGAGGGAU
860	UAUCAGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGGG
862	GUUAUCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGCAG
868	UCCAGAGU	CUGAUGAGGCCGAAAGGCCGAA	AUCAGGAG
872	GCAUCCA	CUGAUGAGGCCGAAAGGCCGAA	AGUUAUCA
883	AGGUUCCA	CUGAUGAGGCCGAAAGGCCGAA	AGGCACUC
894	CTUGGAGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGGUUC
898	AGAUCCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGGACAG
905	GGGUCCCA	CUGAUGAGGCCGAAAGGCCGAA	AUCCUGGA
918	UCACACAU	CUGAUGAGGCCGAAAGGCCGAA	ACUGGGGU
928	CAGAUUCG	CUGAUGAGGCCGAAAGGCCGAA	AUCACACA
934	GAAGGACA	CUGAUGAGGCCGAAAGGCCGAA	AUCUGGAU
938	CAUCGAAG	CUGAUGAGGCCGAAAGGCCGAA	ACAGAUCT
941	UUGCAUCG	CUGAUGAGGCCGAAAGGCCGAA	AGGACAGA
942	AUUGCAUC	CUGAUGAGGCCGAAAGGCCGAA	AAGGACAG
951	AGAGUGCU	CUGAUGAGGCCGAAAGGCCGAA	AUUGCAUC
958	UCCCCUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUGCUCA
972	AAGAACAG	CUGAUGAGGCCGAAAGGCCGAA	AUUUCUCC

973 AAAGAACA CUGAUGAGGCCGAAAGGCCGAA AAUUCUC  
977 CUUUAAAG CUGAUGAGGCCGAAAGGCCGAA ACAGAAU  
978 UCUUUAAG CUGAUGAGGCCGAAAGGCCGAA AACAGAAU  
980 UGUUUUA CUGAUGAGGCCGAAAGGCCGAA AGAACAGA  
981 CUGUCUU CUGAUGAGGCCGAAAGGCCGAA AAGAACAG  
982 CCUGUCU CUGAUGAGGCCGAAAGGCCGAA AAAGAACA  
992 GCCAGAAA CUGAUGAGGCCGAAAGGCCGAA ACCUGUCU  
994 GCGCCAGA CUGAUGAGGCCGAAAGGCCGAA AUACUCU  
995 UGCGCCAG CUGAUGAGGCCGAAAGGCCGAA AAUACCU  
996 UUGCGCCA CUGAUGAGGCCGAAAGGCCGAA AAAUACCU  
1007 UCCUGAGG CUGAUGAGGCCGAAAGGCCGAA ACUUGCGC  
1011 AGAAUCCU CUGAUGAGGCCGAAAGGCCGAA AGGGACUU  
1017 GGUUCGAG CUGAUGAGGCCGAAAGGCCGAA AUCCUGAG  
1018 AGGUUCGA CUGAUGAGGCCGAAAGGCCGAA AAUCCUGA  
1020 UCAGGUUC CUGAUGAGGCCGAAAGGCCGAA AGAAUCCU  
1031 UCAAAUGA CUGAUGAGGCCGAAAGGCCGAA ACUCAGGU  
1032 AUCAAAUG CUGAUGAGGCCGAAAGGCCGAA AACTUCAGG  
1033 GAUCAAU CUGAUGAGGCCGAAAGGCCGAA AAACUCAG  
1036 AGAGAUCA CUGAUGAGGCCGAAAGGCCGAA AUGAAACU  
1037 AAGAGAU CUGAUGAGGCCGAAAGGCCGAA AAUGAAAC  
1041 AAUGAAGA CUGAUGAGGCCGAAAGGCCGAA AUCAAUUG  
1043 AGAAUGAA CUGAUGAGGCCGAAAGGCCGAA AGAUCAAA  
1045 CCAGAAUG CUGAUGAGGCCGAAAGGCCGAA AGAGAUCA  
1046 GCCAGAAU CUGAUGAGGCCGAAAGGCCGAA AAGAGAU C  
1049 AUGGCCAG CUGAUGAGGCCGAAAGGCCGAA AUGAAGAG  
1050 GAUGGCCA CUGAUGAGGCCGAAAGGCCGAA AAUGAAGA  
1058 AAGGAAGA CUGAUGAGGCCGAAAGGCCGAA AUGGCCAG  
1060 UGAAGGAA CUGAUGAGGCCGAAAGGCCGAA AGAUGGCC  
1062 GUCGAAG CUGAUGAGGCCGAAAGGCCGAA AGAGAUUG  
1063 UGCUGAAG CUGAUGAGGCCGAAAGGCCGAA AAGAGAU G  
1066 CACUGCUG CUGAUGAGGCCGAAAGGCCGAA AGGAAGAG  
1067 CCACUGCU CUGAUGAGGCCGAAAGGCCGAA AAGGAAGA  
1085 UAACUUA CUGAUGAGGCCGAAAGGCCGAA AUGCAGCA  
1092 CUGCUAAU CUGAUGAGGCCGAAAGGCCGAA ACUUCUAU  
1093 CCUGCUAA CUGAUGAGGCCGAAAGGCCGAA AACUUAU  
1095 UCCCUGCU CUGAUGAGGCCGAAAGGCCGAA AUUACUUC  
1096 AUCCUUGC CUGAUGAGGCCGAAAGGCCGAA AAUAACUU  
1105 GAAAACAG CUGAUGAGGCCGAAAGGCCGAA AUCCUUGC  
1110 AAAAUGAA CUGAUGAGGCCGAAAGGCCGAA ACAGUAUC  
1111 AAAAUGA CUGAUGAGGCCGAAAGGCCGAA AACAGUAU  
1112 UAAAAUG CUGAUGAGGCCGAAAGGCCGAA AAACAGUA  
1113 UUA AAAAU CUGAUGAGGCCGAAAGGCCGAA AAAACAGU  
1116 CCUUUAAA CUGAUGAGGCCGAAAGGCCGAA AUGAAAA C  
1117 UCCUUUA CUGAUGAGGCCGAAAGGCCGAA AAUGAAAA  
1118 UUCUUUA CUGAUGAGGCCGAAAGGCCGAA AAAUGAAA  
1119 GUUCCUU CUGAUGAGGCCGAAAGGCCGAA AAAAUGAA  
1120 AGUUCUU CUGAUGAGGCCGAAAGGCCGAA AAAAUGA  
1129 CCAGAAU CUGAUGAGGCCGAAAGGCCGAA AGUUCUU  
1133 UGGCCCAG CUGAUGAGGCCGAAAGGCCGAA ACUGAGUU

1134 AUGGCCA CUGAUGAGGCCGAAAGGCCGAA AACUGAGU  
1143 UUUCCUCU CUGAUGAGGCCGAAAGGCCGAA AUGGCCA  
1144 AUUUCCUC CUGAUGAGGCCGAAAGGCCGAA AAUGGCC  
1158 CCAGCUUG CUGAUGAGGCCGAAAGGCCGAA ACCUCAU  
1168 UCUUGGUU CUGAUGAGGCCGAAAGGCCGAA ACCAGCU  
1169 UUCUUGG CUGAUGAGGCCGAAAGGCCGAA AACCAGCU  
1182 AGGGUGUG CUGAUGAGGCCGAAAGGCCGAA AUGCUUCU  
1195 UGAAGGA CUGAUGAGGCCGAAAGGCCGAA ACCCAGG  
1196 UUGAAGG CUGAUGAGGCCGAAAGGCCGAA AACCCAGG  
1197 GUUGAAG CUGAUGAGGCCGAAAGGCCGAA AAACCCAG  
1201 UAUGGUUG CUGAUGAGGCCGAAAGGCCGAA AGGGAAAC  
1202 UUAUGGUU CUGAUGAGGCCGAAAGGCCGAA AAGGAAA  
1209 AUUUUUCU CUGAUGAGGCCGAAAGGCCGAA AUGGUUGA  
1218 GCAGCAUC CUGAUGAGGCCGAAAGGCCGAA AUUUUUCU  
1230 UUAUCAGA CUGAUGAGGCCGAAAGGCCGAA AUGGCAGC  
1231 CUUAUCAG CUGAUGAGGCCGAAAGGCCGAA AAUGGCAG  
1232 CCUUAUCA CUGAUGAGGCCGAAAGGCCGAA AAAUGGCA  
1237 CCUUCCU CUGAUGAGGCCGAAAGGCCGAA AUCAGAAA  
1256 CAAAGAAG CUGAUGAGGCCGAAAGGCCGAA AUGUUUC  
1259 CCACAAAG CUGAUGAGGCCGAAAGGCCGAA AGUAUGUU  
1260 UCCACAAA CUGAUGAGGCCGAAAGGCCGAA AAGUAUGU  
1262 CUUCCACA CUGAUGAGGCCGAAAGGCCGAA AGAAGUAU  
1263 UCUUCCAC CUGAUGAGGCCGAAAGGCCGAA AAGAAGUA  
1277 ACCUCCAG CUGAUGAGGCCGAAAGGCCGAA AUUUGUCU  
1286 UCUCAUC CUGAUGAGGCCGAAAGGCCGAA ACCUCCAG  
1287 UUCUCAUC CUGAUGAGGCCGAAAGGCCGAA AACCUGCA  
1304 GCUCAGG CUGAUGAGGCCGAAAGGCCGAA ACUGUCUC  
1319 GUCUGGA CUGAUGAGGCCGAAAGGCCGAA AGCCUGGC  
1320 UGUCUGG CUGAUGAGGCCGAAAGGCCGAA AAGCCUGG  
1321 AUGUCUG CUGAUGAGGCCGAAAGGCCGAA AAAGCCUG  
1330 UUCUGUA CUGAUGAGGCCGAAAGGCCGAA AUGUCUGG  
1332 UCUUCUG CUGAUGAGGCCGAAAGGCCGAA AUAUGUCU  
1343 UUCUGGA CUGAUGAGGCCGAAAGGCCGAA AGUCUUCU  
1344 AUUCCUG CUGAUGAGGCCGAAAGGCCGAA AAGUCUUC  
1345 AAUCCUG CUGAUGAGGCCGAAAGGCCGAA AAAGUCUU  
1353 UUUGGAU CUGAUGAGGCCGAAAGGCCGAA AUUCCUGG  
1354 CUUUGAU CUGAUGAGGCCGAAAGGCCGAA AAUCCUG  
1357 GAUCUUUG CUGAUGAGGCCGAAAGGCCGAA AUUAUUC  
1365 ACAGCAUC CUGAUGAGGCCGAAAGGCCGAA AUCUUUGG  
1374 GCUUCAA CUGAUGAGGCCGAAAGGCCGAA ACAGCAUC  
1375 UGCUCAA CUGAUGAGGCCGAAAGGCCGAA AACAGCAU  
1376 AUGCUCA CUGAUGAGGCCGAAAGGCCGAA AAACAGCA  
1377 AAUGCUUC CUGAUGAGGCCGAAAGGCCGAA AAAACAGC  
1385 AAAACCA CUGAUGAGGCCGAAAGGCCGAA AUGCUUCA  
1386 AAAAACC CUGAUGAGGCCGAAAGGCCGAA AAUGCUUC  
1391 AAUGAAA CUGAUGAGGCCGAAAGGCCGAA ACCCAAU  
1392 AAUAAGA CUGAUGAGGCCGAAAGGCCGAA AACCCAAA  
1393 GAAAUAGA CUGAUGAGGCCGAAAGGCCGAA AAACCCAA  
1394 AGAAUAG CUGAUGAGGCCGAAAGGCCGAA AAAACCCA

1395	AAGAAUA	CUGAUGAGGCCGAAAGGCCGAA	AAAAACC
1397	UGAAGAA	CUGAUGAGGCCGAAAGGCCGAA	AGAAAAAC
1399	ACUGAAG	CUGAUGAGGCCGAAAGGCCGAA	AUAGAAAA
1400	CACUGAAG	CUGAUGAGGCCGAAAGGCCGAA	AAUAGAAA
1401	CCACUGAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAGAA
1403	AUCCACUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAAUAG
1404	GAUCCACU	CUGAUGAGGCCGAAAGGCCGAA	AAGAAUA
1412	ACUGUGAA	CUGAUGAGGCCGAAAGGCCGAA	AUCCACUG
1414	CGACUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGAUCCAC
1415	CCGACUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGAUCCA
1421	CAAACUCC	CUGAUGAGGCCGAAAGGCCGAA	ACUGUGAA
1427	UUGGGUCA	CUGAUGAGGCCGAAAGGCCGAA	ACUCCGAC
1428	UUUGGGUC	CUGAUGAGGCCGAAAGGCCGAA	AACUCCGA
1458	CUCUUCAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUGUGU
1459	GCUCUUA	CUGAUGAGGCCGAAAGGCCGAA	AACAUGUG
1460	UGCUCUUC	CUGAUGAGGCCGAAAGGCCGAA	AAACAUGU
1478	AACACUGA	CUGAUGAGGCCGAAAGGCCGAA	ACCAGCUG
1479	UAACACUG	CUGAUGAGGCCGAAAGGCCGAA	AACCAGCU
1480	CUAACACU	CUGAUGAGGCCGAAAGGCCGAA	AAACCAGC
1486	CCCCUCCU	CUGAUGAGGCCGAAAGGCCGAA	ACACUGAA
1487	ACCCCUCC	CUGAUGAGGCCGAAAGGCCGAA	AACACUGA
1498	GCCUUCUA	CUGAUGAGGCCGAAAGGCCGAA	ACACCCCU
1500	GUGCCUUC	CUGAUGAGGCCGAAAGGCCGAA	AUACACCC
1519	UCAUUUA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCAU
1520	UUCAUUUA	CUGAUGAGGCCGAAAGGCCGAA	AACAUUA
1521	GUUCAUUU	CUGAUGAGGCCGAAAGGCCGAA	AAACAUUC
1522	GGUUCAUU	CUGAUGAGGCCGAAAGGCCGAA	AAACAUUC
1532	UGAACAAU	CUGAUGAGGCCGAAAGGCCGAA	AGUUAU
1535	UGUGAAC	CUGAUGAGGCCGAAAGGCCGAA	AUUGGUU
1538	AAGUGUUG	CUGAUGAGGCCGAAAGGCCGAA	ACAAUUG
1539	UAAGUGUU	CUGAUGAGGCCGAAAGGCCGAA	AACAAUUA
1546	AAAGUCCU	CUGAUGAGGCCGAAAGGCCGAA	AGUGUGA
1547	CAAAGUCC	CUGAUGAGGCCGAAAGGCCGAA	AAGUGUUG
1553	AACUCACA	CUGAUGAGGCCGAAAGGCCGAA	AGUCCUAA
1554	CAACUCAC	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCUA
1561	GCCACUUC	CUGAUGAGGCCGAAAGGCCGAA	ACUCACAA
1571	GAGAAAAU	CUGAUGAGGCCGAAAGGCCGAA	AGCCACUU
1574	CAGGAGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGAGCCA
1575	GCAGGAGA	CUGAUGAGGCCGAAAGGCCGAA	AAUGAGCC
1576	UGCAGGAG	CUGAUGAGGCCGAAAGGCCGAA	AAAUGAGC
1577	AUGCAGGA	CUGAUGAGGCCGAAAGGCCGAA	AAAUGAG
1579	AUAUGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAAAAUG
1586	UCACAGCA	CUGAUGAGGCCGAAAGGCCGAA	AUGCAGGA
1602	AUGCUCGA	CUGAUGAGGCCGAAAGGCCGAA	AUUCUCAU
1604	UCAUGCUC	CUGAUGAGGCCGAAAGGCCGAA	AGAUUCCC
1620	CAGUUAGA	CUGAUGAGGCCGAAAGGCCGAA	ACACAGUU
1622	UCCAGUUA	CUGAUGAGGCCGAAAGGCCGAA	AUACACAG
1624	AGUCCAGU	CUGAUGAGGCCGAAAGGCCGAA	AGAUACAC
1633	GAUGUGCA	CUGAUGAGGCCGAAAGGCCGAA	AGUCCAGU

1634 CGAUGUGC CUGAUGAGGCCGAAAGGCCGAA AAGUCCAG  
1641 CCCGUAAC CUGAUGAGGCCGAAAGGCCGAA AUGUGCAA  
1644 ACACCCGU CUGAUGAGGCCGAAAGGCCGAA ACGAUGUG  
1645 AACACCCG CUGAUGAGGCCGAAAGGCCGAA AACGAUGU  
1653 CCUGUUUG CUGAUGAGGCCGAAAGGCCGAA ACACCCGU  
1654 GCCUGUUU CUGAUGAGGCCGAAAGGCCGAA AACACCCG  
1670 UGCAAGCU CUGAUGAGGCCGAAAGGCCGAA AGCAGCAG  
1671 GUGCAAGC CUGAUGAGGCCGAAAGGCCGAA AAGCAGCA  
1675 UCAAGUGC CUGAUGAGGCCGAAAGGCCGAA AGCUAAGC  
1681 AUGUGAUC CUGAUGAGGCCGAAAGGCCGAA AGUGCAAG  
1685 UUCAUGU CUGAUGAGGCCGAAAGGCCGAA AUCAAGUG  
1701 UUCUGUG CUGAUGAGGCCGAAAGGCCGAA AGCUCCCU  
1702 GUCUCGUG CUGAUGAGGCCGAAAGGCCGAA AAGCUCCC  
1720 CACAUGAG CUGAUGAGGCCGAAAGGCCGAA ACUUCCCC  
1723 UCACACAU CUGAUGAGGCCGAAAGGCCGAA AGUACUUC  
1744 AUAAGAC CUGAUGAGGCCGAAAGGCCGAA AUCACUCG  
1749 UCCACAUU CUGAUGAGGCCGAAAGGCCGAA ACACAAUC  
1751 AAUCCACA CUGAUGAGGCCGAAAGGCCGAA AGACACAA  
1759 GGGCAAAU CUGAUGAGGCCGAAAGGCCGAA AUCCACAU  
1760 UGGGCAAA CUGAUGAGGCCGAAAGGCCGAA AAUCCACA  
1762 AAUGGGCA CUGAUGAGGCCGAAAGGCCGAA AUAAUCCA  
1763 UAAUGGGC CUGAUGAGGCCGAAAGGCCGAA AAUAUUC  
1770 UAUUAAU CUGAUGAGGCCGAAAGGCCGAA AUGGGCAA  
1771 UUAUUAU CUGAUGAGGCCGAAAGGCCGAA AAUGGGCA  
1773 CUUUUAU CUGAUGAGGCCGAAAGGCCGAA AUAAUGGG  
1774 UCUUUAU CUGAUGAGGCCGAAAGGCCGAA AAUAUUGG  
1775 CUCUUUAU CUGAUGAGGCCGAAAGGCCGAA AAAUAUUG  
1776 AUCCUCUU CUGAUGAGGCCGAAAGGCCGAA AUUAAUAU  
1787 AAUUGACA CUGAUGAGGCCGAAAGGCCGAA AUCCUCUU

Table AVIII: Human Stromelysin Hairpin Ribozyme and Target Sequences

nt. Position	RZ	Substrate
66	GGCAGC AGAA GUAGGA ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UCCUACU GUU GCUUGGCG
82	GCUGAGCA AGAA GCCACG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CGUGGCA GUU UGCUCAGC
91	AUUGGAUA AGAA GAGCAA ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UUGCUCA GCC UAUCCAUU
192	UCCUACCA AGAA GUUACA ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UGAAACA GUU UGUUAGGA
220	UUUUUAC AGAA GGACCA ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UGGUCCU GUU GUUAAAAA
328	UGACCAAC AGAA GGAACU ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AGUUCU GAU GUUGGUCA
412	UUUGGCAA AGAA GGUUGA ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UACACCA GAU UUGCCAAA
430	GCAGAAUC AGAA GCAUCU ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AGAUCCU GUU GAUUCUCC
439	UUUCACAC AGAA GAAUCA ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UGAUUCU GCU GUUGAGAA
442	GCUUUCUC AGAA GCAGAA ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UUUCUCU GUU GAGAAAGC
691	AUUUCAUG AGAA GCNAOG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CGUUGCU GCU CAUGAAAU
775	CGAGUCAG AGAA GGUUGU ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	ACUCACA GAC CUGACTUG
780	GGAAACGA AGAA GGUUCG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CAGACCU GAC UCGGUUCC
786	ACAGGCGG AGAA GAGUCA ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UGACUCG GUU CCGCUCGU
791	UUAGAGACA AGAA GAAACG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CGGUUCC GCC UGUUCUAA
795	CAUCUUGA AGAA GCGGGA ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UCCGCCU GUC UCAAGAUG
822	CAUAGAGG AGAA GAAUGC ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GCAUUCA GUC CCUCUAUG
844	UCAGGGGA AGAA GGGGGA ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UCCCCCU GAC UCCCTUGA
880	UTUGGAGG AGAA GGUUCC ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GGAAACU GUC CCUCCAGA
919	ANGACAA AGAA GGAUCA ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UGAUCCU GCU UUGUCCUU
963	CUUUAAG AGAA GGAUUU ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AAAUCCU GAU CUUUAAG
1360	UTUUCAAA AGAA GCAUCA ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UGAUGCU GUU UUUAGAAG
1407	CAACTUCC AGAA GUGAAG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CUUCACA GUU GGAGUUG
1460	AUUAAACC AGAA GUUACU ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AGUAAACA GCU GGUUAAU
1570	ACAGCACA AGAA GGAGAA ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UUUCUCU GCC UGUUCUGU
1667	AGCUAAGC AGAA GCGCAU ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AUGGGCU GCU GCUUAGCU
1670	GCAAGCUA AGAA GCAGCC ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GGCUGCU GCU UAGCUUGC



Table AIX: Rabbit Hairpin Ribozyme and Target Sequences

nt. Position	Ribozyme Sequence	Substrate
77	CCAGCCAC AGAA GUAGCA ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UGUACU GCU GUUUGUG
99	AGUGGAUA AGAA GAGCAA ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UUUUCA GCC UAUCCACU
203	UUUUAACA AGAA GUUUA ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UGAACA GUU UGUAAAA
231	UUUUUAC AGAA GGAUA ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UAGUCCU GUU GUUAAAA
339	UGUCCAC AGAA GGAUG ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CGUUCCU GAU GUUGUA
423	CUUGCCAG AGAA GGUUG ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CACACG GAU CUUCCAG
441	GCAGCUC AGAA GCAUCU ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	AGAUCCU GUU GAUGUCC
702	AGUUAUG AGAA GCACA ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UGUCCU GCU CAUGACU
731	CCAGUGA AGAA GAUUA ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UGGUCU GUU UCACUCC
758	CUUGUAC AGAA GUGUU ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	ANGGCU GAU GUACCCAG
768	GGUUGUA AGAA GGUUG ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	GUUUAU GUC UACAAAC
786	CGGUCAG AGAA GUUUG ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CUUACA GAC CUUCCCG
797	AAAGGCG AGAA GGGUA ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UGGUCG GUU CGGCUUU
802	UUGAGAA AGAA GAUUG ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CGGUCC GCC UAUUCAA
849	GAGGCGG AGAA GGUUA ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UGGACG GCC CUUCCUC
855	UAGGAGA AGAA GGGUC ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	GGUCCU GCC UCUCUGA
891	CUUGGAG AGAA GGUUC ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	GGAAUCC GUC CUUCCAG
930	ANGGACG AGAA GGAUA ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UGAUCA GAU CUUCCUU
935	CAUGGAG AGAA GAUUG ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CAGAUCC GUC CUUCCAG
974	CUUUAAG AGAA GAUUU ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	AAAUCC GUU CUUUAAG
1107	AAAUAGA AGAA GUUUC ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	GGAUCC GUU UUAUUUU
1130	UGGUCAG AGAA GGUUC ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	GAUUA GUU CUUCCCA
1301	CUUCCAG AGAA GUCUU ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	AGAGCA GUC CUUCCAG
1371	GUUCCAA AGAA GCAUG ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CGAUCC GUU UUGAAGC
1418	CAGAUCC AGAA GUUUG ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CUUACA GUC GGGUUUG
1471	CUGAAAC AGAA GUUCCU ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	AGCAUA GCU GGUUCCAG
1663	AGCUAAG AGAA GGUUG ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	ACAGCCU GCU GCUUAGCU
1666	GGAAGUA AGAA GGAUG ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	GGUCCU GCU UAGCUUC
1732	AUUCACU AGAA GUACA ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UGUGCA GAC GAGUAUU

Table BII: Human B7-1 Hammerhead Ribozyme Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	AAACCCU C UGUAAAG	236	UGUGUGU U UUGUAAA
12	CCUCUGU A AAGUAAAC	237	GUGUGUU U UGUAAAC
17	GUAAAGU A ACAGAAG	238	UGUGUUU U GUAAACA
26	CAGAAGU U AGAAGGG	241	GUUUUGU A AACAUCA
27	AGAAGUU A GAAGGGG	247	UAAACAU C ACUGGAG
41	GAAAGU C GCCUCUC	258	GGAGGGU C UUCUACG
46	GUCGCCU C UCUGAAG	260	AGGGUCU U CUACGUG
48	CGCCUCU C UGAAGAU	261	GGGUCUU C UACGUGA
56	UGAAGAU U ACCCAA	263	GUCUUU A CGUGAGC
57	GAAGAUU A CCCAAAG	274	GAGCAU U GGAUUGU
75	AAGUGAU U UGUCAUU	279	AUUGGAU U GUCAUCA
76	AGUGAUU U GUCAUUG	282	GGAUUGU C AUCAGCC
79	GAUUUGU C AUUGCUU	285	UUGUCAU C AGCCUCU
82	UUGUCAU U GCUUUAU	298	UGCCUGU U UUGCACC
86	CAUUGCU U UAGAGAC	299	GCCUGUU U UGCACCU
87	AUUGCUU U AUAGACU	300	CCUGUUU U GCACCCUG
88	UUGCUUU A UAGACUG	322	CCCUGGU C UUAUUUG
90	GCUUUUA A GACUGUA	324	CUGGUUU U ACTUGGG
97	AGACUGU A AGAAGAG	325	UGGUCUU A CUUGGGU
110	AGAACAU C UCAGAAG	328	UCTUACU U GGGUCCA
112	AACAUCU C AGAAGUG	333	CUUGGGU C CAAAUUG
124	GUGGAGU C UUAACCU	339	UCCAAAU U GUUGGCU
126	GGAGUCU U ACCUGA	342	AAAUUGU U GGCUUUC
127	GAGUCUU A CCCUGAA	347	GUUGGCU U UCACUUU
137	CUGAAAU C AAAGGAU	348	UUGGCUU U CACUUUU
145	AAAGGAU U UAAAGAA	349	UGGCUUU C ACUUUUG
146	AAGGAUU U AAAGAAA	353	UUUCACU U UUGACCC
147	AGGAUUU A AAGAAAA	354	UUCACUU U UGACCCU
163	GUGGAU U UUUCUUC	355	UCACUUU U GACCCUA
164	UGGAUUU U UUCUUA	362	UGACCCU A AGCAUCU
165	GGAAUUU U UCUUCAG	368	UAAGCAU C UGAAGCC
166	GAAUUUU U CUUCAGC	404	GGAACAU C ACCAUCC
167	AAUUUUU C UUCAGCA	410	UCACCAU C CAAGUGU
169	UUUUUCU U CAGCAAG	418	CAAGUGU C CAUACCU
170	UUUUCUU C AGCAAGC	422	UGUCCAU A CCUCAAU
187	UGAAACU A AAUCCAC	426	CAUACCU C AAUUUCU
191	ACTUAAU C CACAACC	430	CCUCAAU U UCUUCA
200	ACAACCU U UGGAGAC	431	CUCAAUU U CUUCAG
201	CAACCUU U GGAGACC	432	UCAAUU C UUUCAGC
221	ACACCCU C CAAUCUC	434	AAUUUCU U UCAGCUC
226	CUCCAAU C UCUGUGU	435	AUUUCUU U CAGCUCU
228	CCAAUCU C UGUGUGU	436	UUUCUUU C AGCUCUU

441	UUCAGCU C UUGGUGC	782	GUGAGCU U AUCAGUC
443	CAGCUCU U GGUGCUG	783	UGACGUU A UCAGUCA
457	GGCUGGU C UUUUCUA	785	ACGUUAU C AGUCAAA
459	CUGGUCU U UCUCACU	789	UAUCAGU C AAAGCUG
460	UGGUCUU U CUCACUU	800	GCUGACU U CCCUACA
461	GGUCUUU C UCACUUC	801	CUGACUU C CUCACAC
463	UCUUUCU C ACUUCUG	805	CUUCCCU A CACCUAG
467	UCUCACU U CUGUUCA	811	UACACCU A GUUAUUC
468	CUCACUU C UGUUCAG	814	ACCUAGU A UAUCUGA
472	CUUCUGU U CAGGUGU	816	CUAGUAU A UCUGACU
473	UUCUGUU C AGGUGUU	818	AGUAUAU C UGACUUU
480	CAGGUGU U AUCCAGG	824	UCUGACU U UGAAAUU
481	AGGUGUU A UCCAGCU	825	CUGACUU U GAUAUUC
483	GUGUUAU C CACGUGA	831	UUGAAAU U CCAACUU
521	ACGCUGU C CUGUGGU	832	UGAAAUU C CAACUUC
529	CUGUGGU C ACAAUUG	838	UCCAACU U CUAUAU
537	ACAAUGU U UCUGUUG	839	CCAACUU C UAAUAUU
538	CAAUGUU U CUGUUGA	841	AACUUCU A AUUAUAG
539	AAUGUUU C UGUUGAA	844	UUCUAU A UUAAGAAG
543	UUUCUGU U GAAGAGC	846	CUAAUAU U AGAAGGA
562	ACAAACU C GCAUCUA	847	UAUAUUU A GAAGGAU
567	CUCGCAU C UACUGGC	855	GAAGGAU A AUUUGCU
569	CGCAUCU A CUGGCAA	858	GGAUAAU U UGCUCAA
601	GCUGACU A UGAUGUC	859	GAUAUUU U GCUCAAC
608	AUGAUGU C UGGGGAC	863	AUUUGCU C AACCUCU
622	CAUGAAU A UAUGGCC	869	UCAACCU C UGGAGGU
624	UGAAUAU A UUGCCCG	877	UGGAGGU U UCCAGA
635	CCCGAGU A CCGAAC	878	GGAGGUU U UCCAGAG
651	GGACCAU C UUGAUUA	879	GAGGUUU U CCAGAGC
653	ACCAUCU U UGAUAUC	880	AGGUUUU C CAGAGCC
654	CCAUCUU U GAUAUCA	889	AGAGCCU C ACCUCUC
658	CUUGAU A UCACUAA	894	CUCACCU C UCCUGGU
660	UUGAUUA C ACUAUA	896	CACUCU C CUGGUUG
664	UAUCACU A AUAACCU	902	UCCUGGU U GGAAAUU
667	CACUAU A ACCUCUC	920	GAAGAAU U AAUGCC
672	AUAACCU C UCCAUUG	921	AAGAAU A AAUGCCA
674	AACCUCU C CAUUGUG	930	AUGCCAU C AACACAA
678	UCUCCAU U GUGAUCC	942	CAACAGU U UCCCAAG
684	UUGUGAU C CUGGCUC	943	AACAGUU U CCCAAGA
691	CCUGGCU C UGCGCCC	944	ACAGUUU C CCAAGAU
701	CGCCCAU C UGACGAG	952	CCAAGAU C CUGAAAC
716	GGCACAU A CGAGUGU	966	CUGAGCU C UAUGCUG
726	AGUGUGU U GUUCUGA	968	GAGCUCU A UGCUGUU
729	GUGUGUU U CUGAAGU	975	AUGCUGU U AGCAGCA
730	UGUUGUU C UGAAGUA	976	UGCUGUU A GCAGCAA
737	CUGAAGU A UGAAAAA	991	ACUGGAAU U UCAAUAU
751	AGACGCU U UCAAGCG	992	CUGGAUU U CAAUAUG
752	GACGCUU U CAAGCGG	993	UGGAUUU C AAUAUGA
753	ACGCUUU C AAGCGGG	997	UUUCAAU A UGACAAC

1016	CACAGCU U CAUGUGU	1315	CAUGGAU C GUGGGGA
1017	ACAGCUU C AUGUGUC	1324	UGGGGAU C AUGAGGC
1024	CAUGUGU C UCAUCAA	1334	GAGGCAU U CUUCCCU
1026	UGUGUCU C AUCAAGU	1335	AGGCAU C UUCCCUU
1029	GUCUCAU C AAGUAUG	1337	GCAUUCU U CCUUAUA
1034	AUCAAGU A UGGACAU	1338	CAUUCU C CUAUAC
1042	UGGACAU U UAAGAGU	1342	CUUCCCU U AACAAAU
1043	GGACAUU U AAGAGUG	1343	UUCCCUU A ACAAAU
1044	GACAUUU A AGAGUGA	1350	AACAAU U UAAGCUG
1054	AGUGAAU C AGACCUU	1351	ACAAU U AAGCUGU
1061	CAGACCU U CAACUGG	1352	CAAAU U AGCUGU
1062	AGACCUU C AACUGGA	1359	AAGCUGU A UUAACCA
1072	CUGGAU A CAACCAA	1360	AGCUGU U UAACCCAC
1090	AGAGCAU U UUCUGA	1361	GCUGUU U ACCCACU
1091	GAGCAU U UCCUGAU	1362	CUGUUU A CCCACUA
1092	AGCAUU U CCUGAUA	1369	ACCCACU A CCUCACC
1093	GCAUUU C CUGAUAA	1373	ACTUACU C ACCUUCU
1099	UCCUGAU A ACCUGCU	1378	CUCACCU U CUUAAAA
1107	ACCUGCU C CCAUCCU	1379	UCACCU C UUAUAAA
1112	CUCCCAU C CUGGGCC	1381	ACCUUCU U AAAAACC
1122	GGGCCAU U ACCUUA	1382	CCUUCU A AAAACCU
1123	GGCCAU A CCUUAU	1390	AAAACCU C UUCAGA
1127	AUUACCU U AAUCUCA	1392	AACUCU U UCAGAU
1128	UUACCU A AUCUCAG	1393	ACCUCU U CAGAUUA
1131	CCUUAU C UCAGUA	1394	CCUUUU C AGAUUA
1133	UUAUCU C AGUAAU	1399	UUCAGAU U AAGCUGA
1137	UCUCAGU A AAUGGAA	1400	UCAGAU A AGCUGAA
1146	AUGGAU U UUGUGA	1412	GAACAGU U ACAAGAU
1147	UGGAUU U UUGUGAU	1413	AACAGU A CAAGAUG
1148	GGAAUU U UGUGAUA	1429	CUGGCAU C CCUCUCC
1149	GAAUUU U GUGAUU	1433	CAUCCU C UCCUUC
1155	UUGUGAU A UGCUGCC	1435	UCCUCU C CUUUCUC
1169	CUGACCU A CUGCUUU	1438	CUCUCCU U UCUCUCC
1175	UACUGCU U UGCCCCA	1439	UCUCCU U CUCUCCA
1176	ACUGCUU U GCCCCA	1440	CUCCUU C UCCCCAU
1214	GAGAGAU U GAGAAGG	1442	CCUUUCU C CCCAUU
1230	AAAGUGU A CGCCUUG	1448	UCCCCAU A UGCAAU
1239	GCCUGU A UAACAGU	1455	AUGCAU U UGCUUA
1241	CCUGUAU A ACAGUGU	1456	UGCAAU U GCUUAU
1249	ACAGUGU C CGCAGAA	1460	AUUUGCU U AAUGUA
1275	AAAAGAU C UGAAGGU	1461	UUUGCU A AUGUAAC
1283	UGAAGGU A GCCUCCG	1466	UUAUGU A ACCUCU
1288	GUAGCCU C CGUCAUC	1471	GUAACCU C UUCUUU
1292	CCUCCGU C AUCUCUU	1473	AACUCU U CUUUUGC
1295	CCGUCAU C UCUUCUG	1474	ACCUCU C UUUUGCC
1297	GUCAUCU C UUCUGGG	1476	CUCUUCU U UUGCCAU
1299	CAUCUCU U CUGGGAU	1477	UCUUCU U UGCCAUG
1300	AUCUCU C UGGGAUA	1478	CUUCUU U GCCAUGU
1307	CUGGGAU A CAUGGAU	1486	GCCAUGU U UCCAUUC

1487	CCAUGUU U CCAUUCU
1488	CAUGUUU C CAUUCUG
1492	UUUCCAU U CUGCCAU
1493	UUCCAUU C UGCCAUC
1500	CUGCCAU C UUGAAUU
1502	GCCAUCU U GAAUUGU
1507	CUUGAAU U GUCUUGU
1510	GAAUUGU C UUGUCAG
1512	AUUGUCU U GUCAGCC
1515	GUCUUGU C AGCCAAU
1523	AGCCAAU U CAUUAUC
1524	GCCAAUU C AUUAUCU
1527	AAUUCAU U AUCUAUU
1528	AUUCAUU A UCUAUUA
1530	UCAUUAU C UAUUAAA
1532	AUUAUCU A UUAACA
1534	UAUCUAU U AAACACU
1535	AUCUAUU A AACACUA
1542	AAACACU A AUUUGAG

228 ACACACA CUGAUGAGGCCGAAAGGCCGAA AGAUUGG  
236 UUUACAA CUGAUGAGGCCGAAAGGCCGAA ACACACA  
237 GUUUACA CUGAUGAGGCCGAAAGGCCGAA AACACAC  
238 UGUUUAC CUGAUGAGGCCGAAAGGCCGAA AAACACA  
241 UGAUGUU CUGAUGAGGCCGAAAGGCCGAA ACAAAC  
247 CUCCAGU CUGAUGAGGCCGAAAGGCCGAA AUGUUUA  
258 CGUAGAA CUGAUGAGGCCGAAAGGCCGAA ACCCUCC  
260 CACGUAG CUGAUGAGGCCGAAAGGCCGAA AGACCCU  
261 UCACGUA CUGAUGAGGCCGAAAGGCCGAA AAGACCC  
263 GCUCACG CUGAUGAGGCCGAAAGGCCGAA AGAAGAC  
274 ACAAUCC CUGAUGAGGCCGAAAGGCCGAA AUUGCTC  
279 UGAUGAC CUGAUGAGGCCGAAAGGCCGAA AUCCAAU  
282 GGCUGAU CUGAUGAGGCCGAAAGGCCGAA ACAAUCC  
285 CAGGGCU CUGAUGAGGCCGAAAGGCCGAA AUGACAA  
298 GGUGCAA CUGAUGAGGCCGAAAGGCCGAA ACAGGCA  
299 AGGUGCA CUGAUGAGGCCGAAAGGCCGAA AACAGGC  
300 CAGGUGC CUGAUGAGGCCGAAAGGCCGAA AAACAGG  
322 CAAGUAA CUGAUGAGGCCGAAAGGCCGAA ACCAGGG  
324 CCCAAGU CUGAUGAGGCCGAAAGGCCGAA AGACCAG  
325 ACCCAAG CUGAUGAGGCCGAAAGGCCGAA AAGACCA  
328 UGGACCC CUGAUGAGGCCGAAAGGCCGAA AGUAAGA  
333 CAAUUUG CUGAUGAGGCCGAAAGGCCGAA ACCCAAG  
339 AGCCAAC CUGAUGAGGCCGAAAGGCCGAA AUUUGGA  
342 GAAAGCC CUGAUGAGGCCGAAAGGCCGAA ACAAUUU  
347 AAAGUGA CUGAUGAGGCCGAAAGGCCGAA AGCCAAC  
348 AAAAGUG CUGAUGAGGCCGAAAGGCCGAA AAGCCAA  
349 CAAAAGU CUGAUGAGGCCGAAAGGCCGAA AAAGCCA  
353 GGGUCAA CUGAUGAGGCCGAAAGGCCGAA AGUGAAA  
354 AGGGUCA CUGAUGAGGCCGAAAGGCCGAA AAGUGAA  
355 UAGGGUC CUGAUGAGGCCGAAAGGCCGAA AAAGUGA  
362 AGAUGCU CUGAUGAGGCCGAAAGGCCGAA AGGGUCA  
368 GGCUUCA CUGAUGAGGCCGAAAGGCCGAA AUGCULA  
404 GGAUGGU CUGAUGAGGCCGAAAGGCCGAA AUGUUCU  
410 ACACUUG CUGAUGAGGCCGAAAGGCCGAA AUGGUGA  
418 AGGUAUG CUGAUGAGGCCGAAAGGCCGAA ACACUUG  
422 AUUGAGG CUGAUGAGGCCGAAAGGCCGAA AUGGACA  
426 AGAAAUU CUGAUGAGGCCGAAAGGCCGAA AGGUUUG  
430 UGAAAGA CUGAUGAGGCCGAAAGGCCGAA AUUGAGG  
431 CUGAAAG CUGAUGAGGCCGAAAGGCCGAA AAUUGAG  
432 GCUGAAA CUGAUGAGGCCGAAAGGCCGAA AAAUUGA  
434 GAGCTUG CUGAUGAGGCCGAAAGGCCGAA AGAAAUU  
435 AGAGCTG CUGAUGAGGCCGAAAGGCCGAA AAGAAAU  
436 AAGAGCU CUGAUGAGGCCGAAAGGCCGAA AAAGAAA  
441 GCACCAA CUGAUGAGGCCGAAAGGCCGAA AGCTUGA  
443 CAGCACC CUGAUGAGGCCGAAAGGCCGAA AGAGCTG  
457 UGAGAAA CUGAUGAGGCCGAAAGGCCGAA ACCAGCC  
459 AGUGAGA CUGAUGAGGCCGAAAGGCCGAA AGACCAG  
460 AAGUGAG CUGAUGAGGCCGAAAGGCCGAA AAGACCA  
461 GAAGUGA CUGAUGAGGCCGAAAGGCCGAA AAAGACT

463	CAGAAGU	CUGAUGAGGCCGAAAGGCCGAA	AGAAAGA
467	UGAACAG	CUGAUGAGGCCGAAAGGCCGAA	AGUGAGA
468	CUGAACA	CUGAUGAGGCCGAAAGGCCGAA	AAGUGAG
472	ACACCTG	CUGAUGAGGCCGAAAGGCCGAA	ACAGAAG
473	AACACCU	CUGAUGAGGCCGAAAGGCCGAA	AACAGAA
480	CGUGGAU	CUGAUGAGGCCGAAAGGCCGAA	ACACCTG
481	ACGUGGA	CUGAUGAGGCCGAAAGGCCGAA	AACACCU
483	UCACGUG	CUGAUGAGGCCGAAAGGCCGAA	AUAACAC
521	ACCACAG	CUGAUGAGGCCGAAAGGCCGAA	ACAGCGU
529	ACAUUGU	CUGAUGAGGCCGAAAGGCCGAA	ACCACAG
537	CAACAGA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUGU
538	UCAACAG	CUGAUGAGGCCGAAAGGCCGAA	AACAUUG
539	UUAACA	CUGAUGAGGCCGAAAGGCCGAA	AAACAUU
543	GCUCUUC	CUGAUGAGGCCGAAAGGCCGAA	ACAGAAA
562	UAGAUGC	CUGAUGAGGCCGAAAGGCCGAA	AGUUUGU
567	GCCAGUA	CUGAUGAGGCCGAAAGGCCGAA	AUGCGAG
569	UUGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGCG
601	GACAUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUCAGC
608	GUCCCCA	CUGAUGAGGCCGAAAGGCCGAA	ACAUCAU
622	GGCCAUU	CUGAUGAGGCCGAAAGGCCGAA	AUUCAUG
624	CGGGCCA	CUGAUGAGGCCGAAAGGCCGAA	AUAUUA
635	GUUCUUG	CUGAUGAGGCCGAAAGGCCGAA	ACUCGGG
651	UAUCAA	CUGAUGAGGCCGAAAGGCCGAA	AUGGUCC
653	GAUAUCA	CUGAUGAGGCCGAAAGGCCGAA	AGAUGGU
654	UGAUUUC	CUGAUGAGGCCGAAAGGCCGAA	AAGAUGG
658	UUAGUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCAAAG
660	UAUUAGU	CUGAUGAGGCCGAAAGGCCGAA	AUAUCAA
664	AGGUUAU	CUGAUGAGGCCGAAAGGCCGAA	AGUGAUA
667	GAGAGGU	CUGAUGAGGCCGAAAGGCCGAA	AUUAGUG
672	CAAUGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGUUAU
674	CACAAUG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGUU
678	GUAUCAC	CUGAUGAGGCCGAAAGGCCGAA	AUGGAGA
684	GAGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCACAA
691	GGCCGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCCAGG
701	CUCGUCA	CUGAUGAGGCCGAAAGGCCGAA	AUGGGCG
716	ACACUCG	CUGAUGAGGCCGAAAGGCCGAA	AUGUGCC
726	UCAGAAC	CUGAUGAGGCCGAAAGGCCGAA	ACACACU
729	ACTUCAG	CUGAUGAGGCCGAAAGGCCGAA	ACAACAC
730	UACUUCA	CUGAUGAGGCCGAAAGGCCGAA	AACAACA
737	UUUUUCA	CUGAUGAGGCCGAAAGGCCGAA	ACTUCAG
751	CGCUUGA	CUGAUGAGGCCGAAAGGCCGAA	AGCGUCU
752	CCGCUUG	CUGAUGAGGCCGAAAGGCCGAA	AAGCGUC
753	CCCGCUU	CUGAUGAGGCCGAAAGGCCGAA	AAAGCGU
782	GACUGAU	CUGAUGAGGCCGAAAGGCCGAA	ACGUCAC
783	UGACUGA	CUGAUGAGGCCGAAAGGCCGAA	AACGUCA
785	UUUGACU	CUGAUGAGGCCGAAAGGCCGAA	AUAACGU
789	CAGCUUU	CUGAUGAGGCCGAAAGGCCGAA	ACUGAUA
800	UGUAGGG	CUGAUGAGGCCGAAAGGCCGAA	AGUCAGC
801	GUGUAGG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCAG

805	CUAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AGGGAAG
811	GAUAUAC	CUGAUGAGGCCGAAAGGCCGAA	AGGUGUA
814	UCAGAUU	CUGAUGAGGCCGAAAGGCCGAA	ACTUAGGU
816	AGUCAGA	CUGAUGAGGCCGAAAGGCCGAA	AUAUAG
818	AAAGUCA	CUGAUGAGGCCGAAAGGCCGAA	AUAUACU
824	AAUUUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUCAGA
825	GAUUUC	CUGAUGAGGCCGAAAGGCCGAA	AAGUCAG
831	AAGUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUUCA
832	GAAGUUG	CUGAUGAGGCCGAAAGGCCGAA	AAUUUCA
838	AUAUAG	CUGAUGAGGCCGAAAGGCCGAA	AGUUGA
839	AAUAUA	CUGAUGAGGCCGAAAGGCCGAA	AAGUUG
841	CUAAUAU	CUGAUGAGGCCGAAAGGCCGAA	AGAAGUU
844	CUUCUAA	CUGAUGAGGCCGAAAGGCCGAA	AUUAGAA
846	UCCUUCU	CUGAUGAGGCCGAAAGGCCGAA	AUAUAG
847	AUCCUUC	CUGAUGAGGCCGAAAGGCCGAA	AAUAUA
855	AGCAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUCCUUC
858	UUGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AUUAUCC
859	GUUGAGC	CUGAUGAGGCCGAAAGGCCGAA	AAUAUC
863	AGAGGUU	CUGAUGAGGCCGAAAGGCCGAA	AGCAAAU
869	ACCUCCA	CUGAUGAGGCCGAAAGGCCGAA	AGGUUGA
877	UCUGGAA	CUGAUGAGGCCGAAAGGCCGAA	ACCUCCA
878	CUCUGGA	CUGAUGAGGCCGAAAGGCCGAA	AACCUCC
879	GCTUCUG	CUGAUGAGGCCGAAAGGCCGAA	AAACCTUC
880	GGCTUCG	CUGAUGAGGCCGAAAGGCCGAA	AAAACCTU
889	GAGAGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGCTUCU
894	ACCAGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGUGAG
896	CAACCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGUG
902	AUUUUCC	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGA
920	GGCAUUU	CUGAUGAGGCCGAAAGGCCGAA	AUUCUUC
921	UGGCAUU	CUGAUGAGGCCGAAAGGCCGAA	AAUUCUU
930	UUGUGUU	CUGAUGAGGCCGAAAGGCCGAA	AUGGCAU
942	CUUGGGA	CUGAUGAGGCCGAAAGGCCGAA	ACTUGUG
943	UCUUGGG	CUGAUGAGGCCGAAAGGCCGAA	AACUGUU
944	AUCUUGG	CUGAUGAGGCCGAAAGGCCGAA	AAACUGU
952	GUUUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUUGG
966	CAGCAUA	CUGAUGAGGCCGAAAGGCCGAA	AGCUCAG
968	AACAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGAGCUC
975	UGCUGCU	CUGAUGAGGCCGAAAGGCCGAA	ACAGCAU
976	UUGCUGC	CUGAUGAGGCCGAAAGGCCGAA	AACAGCA
991	AUAUUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCCAGU
992	CAUAUUG	CUGAUGAGGCCGAAAGGCCGAA	AAUCCAG
993	UCAUAUU	CUGAUGAGGCCGAAAGGCCGAA	AAAUCCA
997	GUUGUCA	CUGAUGAGGCCGAAAGGCCGAA	AUUGAAA
1016	ACACAUG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGUG
1017	GACACAU	CUGAUGAGGCCGAAAGGCCGAA	AAGCUGU
1024	UUGAUGA	CUGAUGAGGCCGAAAGGCCGAA	ACACAUG
1026	ACTUGAU	CUGAUGAGGCCGAAAGGCCGAA	AGACACA
1029	CAUACTU	CUGAUGAGGCCGAAAGGCCGAA	AUGAGAC
1034	AUGUCCA	CUGAUGAGGCCGAAAGGCCGAA	ACTUGAU



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1043 CACUCUU CUGAUGAGGCCGAAAGGCCGAA AAUGUCC  
1044 UCAUCUU CUGAUGAGGCCGAAAGGCCGAA AAAUGUC  
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1090 UCAGGAA CUGAUGAGGCCGAAAGGCCGAA AUGCUCU  
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1092 UAUCAGG CUGAUGAGGCCGAAAGGCCGAA AAAUGCU  
1093 UUAUCAG CUGAUGAGGCCGAAAGGCCGAA AAAAUGC  
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1122 UUAAGGU CUGAUGAGGCCGAAAGGCCGAA AUGGCC  
1123 AUUAAGG CUGAUGAGGCCGAAAGGCCGAA AAUGGCC  
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1131 UUAUGA CUGAUGAGGCCGAAAGGCCGAA AUUAAGG  
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1307 AUCCAUG CUGAUGAGGCCGAAAGGCCGAA AUCCCAG  
1315 UCCCCAC CUGAUGAGGCCGAAAGGCCGAA AUCCAUG  
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1335 AAGGGAA CUGAUGAGGCCGAAAGGCCGAA AAUGCUC  
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1338 GUUAAGG CUGAUGAGGCCGAAAGGCCGAA AAGAAUG

1342 AUUUGUU CUGAUGAGGCCGAAAGGCCGAA AGGGAAG  
1343 AAUUGU CUGAUGAGGCCGAAAGGCCGAA .AAGGGAA  
1350 CAGCUUA CUGAUGAGGCCGAAAGGCCGAA AUUUGUU  
1351 ACAGCTU CUGAUGAGGCCGAAAGGCCGAA AAUUGU  
1352 AACAGCU CUGAUGAGGCCGAAAGGCCGAA AAAUUG  
1359 UGGGUA CUGAUGAGGCCGAAAGGCCGAA ACAGCTU  
1360 GUGGGUA CUGAUGAGGCCGAAAGGCCGAA AACAGCU  
1361 AGUGGU CUGAUGAGGCCGAAAGGCCGAA AAACAGC  
1362 UAGUGG CUGAUGAGGCCGAAAGGCCGAA AAAACAG  
1369 GGUGAG CUGAUGAGGCCGAAAGGCCGAA AGUGGU  
1373 AGAAGGU CUGAUGAGGCCGAAAGGCCGAA AGGUAGU  
1378 UUUUAG CUGAUGAGGCCGAAAGGCCGAA AGGUGAG  
1379 UUUUUA CUGAUGAGGCCGAAAGGCCGAA AAGGUGA  
1381 GGUUUU CUGAUGAGGCCGAAAGGCCGAA AGAAGGU  
1382 AGGUUU CUGAUGAGGCCGAAAGGCCGAA AAGAAGG  
1390 UCUGAA CUGAUGAGGCCGAAAGGCCGAA AGGUUU  
1392 AAUCUGA CUGAUGAGGCCGAAAGGCCGAA AGAGGUU  
1393 UAAUCUG CUGAUGAGGCCGAAAGGCCGAA AAGAGGU  
1394 UUAUCU CUGAUGAGGCCGAAAGGCCGAA AAAGAGG  
1399 UCAGCTU CUGAUGAGGCCGAAAGGCCGAA AUCUGAA  
1400 UUCAGCTU CUGAUGAGGCCGAAAGGCCGAA AAUCUGA  
1412 AUCUUG CUGAUGAGGCCGAAAGGCCGAA ACUGUUC  
1413 CAUCUUG CUGAUGAGGCCGAAAGGCCGAA AACUGUU  
1429 GGAGAG CUGAUGAGGCCGAAAGGCCGAA AUGCCAG  
1433 GAAAGGA CUGAUGAGGCCGAAAGGCCGAA AGGGAUG  
1435 GAGAAAG CUGAUGAGGCCGAAAGGCCGAA AGAGGGA  
1438 GGGGAGA CUGAUGAGGCCGAAAGGCCGAA AGGAGAG  
1439 UGGGGAG CUGAUGAGGCCGAAAGGCCGAA AAGGAGA  
1440 AUGGGGA CUGAUGAGGCCGAAAGGCCGAA AAAGGAG  
1442 AUAUGG CUGAUGAGGCCGAAAGGCCGAA AGAAAGG  
1448 AAUUGCA CUGAUGAGGCCGAAAGGCCGAA AUGGGGA  
1455 UUAAGCA CUGAUGAGGCCGAAAGGCCGAA AUUGCAU  
1456 AUUAAGC CUGAUGAGGCCGAAAGGCCGAA AAUUGCA  
1460 UUAACAU CUGAUGAGGCCGAAAGGCCGAA AGCAAAU  
1461 GUUACAU CUGAUGAGGCCGAAAGGCCGAA AAGCAA  
1466 AAGAGGU CUGAUGAGGCCGAAAGGCCGAA ACAUUA  
1471 AAAAGAA CUGAUGAGGCCGAAAGGCCGAA AGGUUAC  
1473 GCAAAAG CUGAUGAGGCCGAAAGGCCGAA AGAGGUU  
1474 GGCAAAA CUGAUGAGGCCGAAAGGCCGAA AAGAGGU  
1476 AUGGCA CUGAUGAGGCCGAAAGGCCGAA AGAAGAG  
1477 CAUGGCA CUGAUGAGGCCGAAAGGCCGAA AAGAAGA  
1478 ACAUGGC CUGAUGAGGCCGAAAGGCCGAA AAGAAG  
1486 GAAUGGA CUGAUGAGGCCGAAAGGCCGAA ACAUGGC  
1487 AGAAUGG CUGAUGAGGCCGAAAGGCCGAA AACAUUG  
1488 CAGAAUG CUGAUGAGGCCGAAAGGCCGAA AAACAUG  
1492 AUGGCAG CUGAUGAGGCCGAAAGGCCGAA AUGGAAA  
1493 GAUGGCA CUGAUGAGGCCGAAAGGCCGAA AAUGGAA  
1500 AAUUCAA CUGAUGAGGCCGAAAGGCCGAA AUGGCAG  
1502 ACAAUUC CUGAUGAGGCCGAAAGGCCGAA AGAUGGC

1507	ACAAGAC	CUGAUGAGGCCGAAAGGCCGAA	AUUCAAG
1510	CUGACAA	CUGAUGAGGCCGAAAGGCCGAA	ACAAUUC
1512	GGCUGAC	CUGAUGAGGCCGAAAGGCCGAA	AGACAAU
1515	AUUGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACAAGAC
1523	GAUAAUG	CUGAUGAGGCCGAAAGGCCGAA	AUUGGCU
1524	AGAUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGC
1527	AAUAGAU	CUGAUGAGGCCGAAAGGCCGAA	AUGAAU
1528	UAAUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAUGAAU
1530	UUUAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAAUGA
1532	UGUUUAA	CUGAUGAGGCCGAAAGGCCGAA	AGAUAAU
1534	AGUGUUU	CUGAUGAGGCCGAAAGGCCGAA	AUAGAU
1535	UAGUGUU	CUGAUGAGGCCGAAAGGCCGAA	AAUAGAU
1542	CUCAAAU	CUGAUGAGGCCGAAAGGCCGAA	AGUGUUU

Table BIV: Mouse B7-1 Hammerhead Ribozyme Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	GaGUuUU a UACcUcA	108	CaUcUUU a GCAuCUG
10	guUuuAU A CCUCAAU	108	CAUcUUU a gcaUCUG
10	GUuUaU a ccuCAAU	131	aUGCCAU C caGgcUU
14	uAUacCU c aAUAGAC	142	gCUuCUU U uUCuaCA
18	CcucAAU A gaCUCUu	142	gCUUCUU u UUCUaCa
18	CCUCaaU a gaCUCUU	143	CUuCUUU u UCuaCAU
18	CcUCAAU a GaCUcuU	143	CuUcUuU u uCUaCAU
23	AuaGaCU c uUAaG	143	CUUCUUU U uCUaCaU
25	AGACuCU U aCUAGuu	143	cUUCuUU u UCUAcau
26	GACuCUU a CuAGuuu	144	UuCuUuU U cUaCAuC
29	UCTUACU a GuuUCuc	144	UuCuuuU u cUAACUC
29	UcUuACU a gUuuCuC	144	UUCuuUU u cuaCAUC
29	UCUuACU a guUUCUC	147	uUUUuCU a cAuCUCU
29	UCuuacu a gUUCUC	153	uAcAuCU C ugUUUCU
34	CUaGUuU c UCUuuuU	165	uUCUGAU U UuUgUGA
34	CUAGUuU c UCUuuuU	165	uCUcgAU u UuuGUGA
34	cUAguuU c uCuUuUU	165	ucucgAU U UUUGUGA
40	ucuCUuU U UCAGgUU	166	CUCgAUU U uUgUGAG
41	cUCUuUU u caGGuUg	167	uCGAUuU u UGUGAGc
41	cuCUuUU U CAGgUUg	167	ucGauUU U UGUGAgC
42	uCUuUUU C AGgUUgu	167	UCgAUUU u UgUGAGC
56	UGAAACU c AAcCuuc	168	cGAUuUu u gUgAGCC
56	UGAAAcU C aAcCUUC	168	cgAUUUU U GUGAgcc
62	uCAACCU U caaAGAC	197	GCUccAU u GgCUcUA
62	UCaAcCU U CaaAGac	202	aUUGGCU c UagaUUc
62	UCAACCU u caaAGac	208	UCuAgAU U ccUGGCU
63	CAACCTU c aaAGACa	216	CCUGGCU u UcCcCau
73	aGAcAcU c UGuUCCa	217	cUGGCUU U CcCcaUc
77	acUCUgU u cCAuUUC	217	cUgGCUu u CccCAUC
78	CucUGUU c CauUUCU	217	CUGGCUu u CCcCauC
83	UucCAuU U CUGUgga	218	UGGcuUU c ccCaUCA
93	GUggAcU A AuAGgAu	218	UGGCUUU C cCcaUca
93	gUgGacU a AUAGgaU	218	UGgCuUU c cCcaUCA
93	gUGgAcU a AuAGGAU	218	ugGcUUU c CCCAUca
96	GAcuAAU a GGAUcaU	224	UCcCCAU c aUGuUCu
96	gacuAAU a gGAUCaU	224	UccCCAU c aUGuucU
101	AUaGGAU c aUCuUuA	230	UCAugUU C UccAAAg
104	GGAUCAU C uuuAgCa	232	AuGUUcU C CAAAGCa
104	GGAUCAU C UUUagca	232	AUGuUcU c caaAGCA
106	AuCAUcU U UagcAUC	232	AugUUCU c cAAAGCa
107	UcAuCuU u AGCAUCU	241	AAAGcAU c UgAAGcu
107	uCaUCUU u AgcAuCU	241	aAAGCAU C UGAAGCU

241	AAAgcAU C UGAAGcU	556	ACCUACU c uCUuAuc
249	UGAAGcU A UGGCuUG	556	AcCUAcU c ucUUAUC
264	CAAuUgU c AGuUGaU	560	AcUcUcU U aUCAuCC
287	CACCaCU c CUcaagU	561	cUCuCUU a UcAuCCU
295	CUCaAgU u UCCaUGU	561	cuCUcuU a uCAUCCU
295	cuCAaGU U UCCAUGu	561	CUCUCuU a UCauCCu
296	uCAAGuU u ccAUgUc	566	UUaUcAU C CUGGgcC
297	CAAGUuU C CAUgUcC	566	uUauCAU C CUGGGCC
297	CAAGuuU c cAUgUCC	581	UGGuCcU U UcAGAcc
314	GGCUcaU u cUUCUcU	583	guCCUUU C AgaCcGG
314	GgcUcAU U CUUCuCU	583	GuCcUUU c AGAccGg
315	GcuCAUu c UuCUcuU	598	GGCACAU A CagcUGU
315	gcuCAUu C UUCuCUU	608	gcUGUGU c GUUCaaA
317	uCAUUCU U CuCUUug	611	GUGUcgU u CAaaaGA
318	CAUUCUu C uCUUugu	611	GUGUcGU U CaaAAGa
318	CAUuCuU C UCuUUgu	612	UGUcGUU C aaAAGaA
320	uUCUUCU c uuUGuGC	641	aUGaAGU u aaACaCU
320	UUCuuCU C UUuGUGC	649	AAAcacU U GGCTUUa
322	CuuCUCU U uGUGCUG	649	AaaCaCU U gGCTUUa
322	CUucUcU u UgUGCUG	655	UUggcuU u AGUAAAg
323	UUCuCUU u gUGcugC	656	UGgcUUU a GUAAAgU
336	gcUGAUU c GUcUUCc	659	CuUuaGU A AAGUuGu
341	uUCGuCU u UCacAAG	664	GUaAaGU U gUCcaUC
341	UUCgucU u UCacAAG	667	AaGUUgU c caUCAAA
342	UcGUCUU U CaCAagU	671	UgUCcaU C AAAGCUG
343	cgucUuU C AcaAGUG	682	gCUgAcU u CuCuACC
343	cGuCuUU c AcaAGUG	682	GCTUGACU U CuCUACc
352	caAGUGU C uuCAGAu	682	GCTUGacU U cuCuACc
355	gUgUcUU C AGaUGUU	683	CUGACUU C uCUACcC
382	UCcaAGU c AgUGaAA	683	CUGACUU c ucuAccC
408	gCUGCcU U GCCguuA	685	gACUuCU c UaCCCCc
414	UUGccgU U aCAACUc	685	gaCUucU c UACCCcC
414	UUgCCgU u ACAAcUc	687	CUUCuCU A CcCCcAa
421	UaCAAcU c uCcUcAU	698	ccAACAU a ACUGagu
426	CUcUCCU c aUgAAgA	698	CCaacAU A ACuGaGU
439	GaUGAgU c UGAaGaC	718	AAcCCaU C UGcAgAc
452	acCGaAU C UACUGGC	718	aaCCCAU c UGcAgac
454	CGaAUcU A CUGGCAA	729	AGACacU A AaAgGAU
484	GuGCUgU c UGucaUU	729	agAcAcU A aaAGGAU
484	GugCUGU c UguCAuU	729	agACAcU a AaAgGAU
488	ugUcUGU C AUUGCUg	737	aaAGGAU u AccUGCU
503	gGAAacU A aaAGuGu	737	aaAGGAU U AccUGCu
503	ggAAAcU a AAagUGU	737	aaagGAU u ACCUGCU
520	CCCGAGU A uAAGAAC	745	accUGcU U UGCuuCc
535	cGGAcUU U aUaUGAc	745	accUGcU u UGCUuCC
536	GGAcUUU a UaUGAcA	759	cGggGgU U uCCCAAA
538	AcUuUAU a UGACaac	759	cGgGGGU u UcCcAaa
553	acuACCU a cUCUcUU	759	cGgGGGU U UcCCaAA
553	AcUaCCU a cUCUcUU	760	GggGgUU u CCCAAAG

760	gGGgGUU u cCCAAag	1060	AAAUgcU u cUGUaAG
760	GGgGGUU U cCCAAAG	1060	AAAUGC U u cUGUaAG
761	GgGGUUU c CCAaAGC	1061	AAUGeUU C UGUaagc
771	aAAgcccU C GCuUCUC	1080	AagcugU u UCAGAAG
771	AaAGCCU C gCuUCUC	1080	AAGCUGU U UcAgaag
776	CUCgCUU C UcUUggu	1081	AgCuGUU u CAgAga
776	CUCgCuU C UCuUGGU	1121	acAGcCU U ACCuUcg
778	CgCuUCU C uUGGUUG	1121	AcAgCCU u ACCuUcG
784	UCuUGGU U GGAAAU	1121	ACagCCU u ACCUUCg
803	GAGaaUU A CCugGcA	1122	CaGcCuU a cCUUCgG
803	gAGAAUU A ccUGgCA	1126	CUuACCU u CgGgccU
803	gagAaUU a CCUGgcA	1127	UUaCcUU c ggGcCUG
812	cUGgCAU C AAuACgA	1127	UUaCcUU c GggCCUg
812	CUGGCAU c aAuaCgA	1144	GaagCAU U AgCUGAA
816	caUCAAU A cGACAAu	1144	gaAGcaU u AGCUGAA
816	cAUCAAU a cgACAAU	1145	aAgCAUU a GCUgAAC
824	CgACAAU U UCCCagG	1160	AGAcCgU c UUCUUuu
825	gACAAUU U CCCagGA	1162	AcCgUCU u CcUUuaG
826	ACAAUUU C CCagGAU	1163	ccGUCUU c CUUuaGU
834	CCagGAU C CUGAAuC	1167	cUUCcUU u AGuUCUU
841	CcUGaaU C ugAAUUG	1177	uUCUUU c UguCCAU
841	cCUGAAU c UGAaUUG	1181	UCuCuGU C CAUGUGg
850	gAAuUGU A CaCCaUu	1181	ucUCUGU c CAUGUGg
869	gccAaCU a gAUuUCA	1192	gUGGGAU A CAUGGua
869	GCCAaCU a GAUuUca	1199	aCaUGGU a UUAUGUG
869	GCCAaCU a gaUuUCA	1201	AuGgUaU u aUGUGGc
873	acUaGAU u UCAaUAc	1210	ugUGGcU C aUGAGGu
873	ACUaGAU U UCAAUAc	1210	UGuGGcU C AUGAGGu
874	CUaGAUU U CAAUAcG	1223	GUacAAU c UUUCUUu
875	UaGAUUU C AAUAcGA	1225	ACAAUcU U UCUuUca
885	UAcgACU c gCAACCa	1225	ACAAuCU u uCuUuca
899	ACACCaU u aAgUgUC	1226	caAuCUU u cUuUCAG
899	ACAcCaU u AaGUGUC	1227	aAucUUU c uUUCAGC
906	UaaGUGU c UcaUuAA	1227	AAucuuU C UUUCAGc
906	uAaGUGU C UCAUuAA	1227	AAuCUuU c uUucaGC
908	agUGUCU C AUuAAaU	1229	ucUUUCU U UCAGCaC
911	GUCUCAU u AAaUAUG	1230	cUUUCUU U CAGCaCc
916	AUuAAaU a UGGaGAU	1252	cUgAUcU u UcgGACA
916	AUuAAaU A UGGaGAU	1274	acaAGAU a gAGuUaA
943	gAGgaCU U CAcTUGG	1310	UGAgGaU u cCuUuCc
944	AGgaCUU C AccUGGg	1312	aGgAUUU c UuUcCAu
1001	UGCUCUU u GggGCAG	1314	gAUUUcU u UcCAUCA
1034	CAGucGU c gUCAuCG	1316	UUUcUuU c CAUCagG
1037	UcGUCgU C AuCguUG	1320	UUUcCaU C AGgAAGC
1043	UCAUCgU U GuCAUCA	1320	UUUCcaU c aggaAGC
1046	ucgUUGU c AuCAUCA	1339	GgCAagU u UgCUGGG
1049	uUguCaU c AuCAAU	1355	cUuUgaU U GCUUgAA
1060	aaaUGcU U CUGUaag	1437	gUGguaU A aGAAaaa
1060	AAaUGCU u cUGUaAG	1437	gUggUaU a AGAAaaa

Table BV: M use B7-1 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequences
8	UGAGGUA CUGAUGAGGCCGAAAGGCCGAA AAAACUC
10	AUUGAGG CUGAUGAGGCCGAAAGGCCGAA AUAAAAC
10	AUUGAGG CUGAUGAGGCCGAAAGGCCGAA AUAAAAC
14	GUCUAUU CUGAUGAGGCCGAAAGGCCGAA AGGUAUA
18	AAGAGUC CUGAUGAGGCCGAAAGGCCGAA AUUGAGG
18	AAGAGUC CUGAUGAGGCCGAAAGGCCGAA AUUGAGG
18	AAGAGUC CUGAUGAGGCCGAAAGGCCGAA AUUGAGG
23	CUAGUAA CUGAUGAGGCCGAAAGGCCGAA AGUCUAU
25	AACUAGU CUGAUGAGGCCGAAAGGCCGAA AGAGUCU
26	AAACUAG CUGAUGAGGCCGAAAGGCCGAA AAGAGUC
29	GAGAAAC CUGAUGAGGCCGAAAGGCCGAA AGUAAGA
29	GAGAAAC CUGAUGAGGCCGAAAGGCCGAA AGUAAGA
29	GAGAAAC CUGAUGAGGCCGAAAGGCCGAA AGUAAGA
29	GAGAAAC CUGAUGAGGCCGAAAGGCCGAA AGUAAGA
34	AAAAAGA CUGAUGAGGCCGAAAGGCCGAA AAACUAG
34	AAAAAGA CUGAUGAGGCCGAAAGGCCGAA AAACUAG
34	AAAAAGA CUGAUGAGGCCGAAAGGCCGAA AAACUAG
40	AACCUGA CUGAUGAGGCCGAAAGGCCGAA AAAGAGA
41	CAACCUG CUGAUGAGGCCGAAAGGCCGAA AAAAGAG
41	CAACCUG CUGAUGAGGCCGAAAGGCCGAA AAAAGAG
42	ACAACCU CUGAUGAGGCCGAAAGGCCGAA AAAAGA
56	GAAGGUU CUGAUGAGGCCGAAAGGCCGAA AGUUUCA
56	GAAGGUU CUGAUGAGGCCGAAAGGCCGAA AGUUUCA
62	GUCUUUG CUGAUGAGGCCGAAAGGCCGAA AGGUUGA
62	GUCUUUG CUGAUGAGGCCGAAAGGCCGAA AGGUUGA
62	GUCUUUG CUGAUGAGGCCGAAAGGCCGAA AGGUUGA
63	UGUCUUU CUGAUGAGGCCGAAAGGCCGAA AAGGUUG
73	UGGAACA CUGAUGAGGCCGAAAGGCCGAA AGUGUCU
77	GAAAUUG CUGAUGAGGCCGAAAGGCCGAA ACAGAGU
78	AGAAAUU CUGAUGAGGCCGAAAGGCCGAA AACAGAG
83	UCCACAG CUGAUGAGGCCGAAAGGCCGAA AAUGGAA
93	AUCCUAU CUGAUGAGGCCGAAAGGCCGAA AGUCCAC
93	AUCCUAU CUGAUGAGGCCGAAAGGCCGAA AGUCCAC
93	AUCCUAU CUGAUGAGGCCGAAAGGCCGAA AGUCCAC
96	AUGAUCC CUGAUGAGGCCGAAAGGCCGAA AUUAGUC
96	AUGAUCC CUGAUGAGGCCGAAAGGCCGAA AUUAGUC
101	UAAAGAU CUGAUGAGGCCGAAAGGCCGAA AUCCUAU
104	UGCUTAA CUGAUGAGGCCGAAAGGCCGAA AUGAUCC
104	UGCUTAA CUGAUGAGGCCGAAAGGCCGAA AUGAUCC
106	GAUGCUA CUGAUGAGGCCGAAAGGCCGAA AGAUGAU

107 AGAUGCU CUGAUGAGGCCGAAAGGCCGAA AAGAUGA  
107 AGAUGCU CUGAUGAGGCCGAAAGGCCGAA AAGAUGA  
108 CAGAUGC CUGAUGAGGCCGAAAGGCCGAA AAAGAUG  
108 CAGAUGC CUGAUGAGGCCGAAAGGCCGAA AAAGAUG  
131 AAGCCUG CUGAUGAGGCCGAAAGGCCGAA AUGGCAU  
142 UGUAGAA CUGAUGAGGCCGAAAGGCCGAA AAGAAGC  
142 UGUAGAA CUGAUGAGGCCGAAAGGCCGAA AAGAAGC  
143 AUGUAGA CUGAUGAGGCCGAAAGGCCGAA AAAGAAG  
143 AUGUAGA CUGAUGAGGCCGAAAGGCCGAA AAAGAAG  
143 AUGUAGA CUGAUGAGGCCGAAAGGCCGAA AAAGAAG  
143 AUGUAGA CUGAUGAGGCCGAAAGGCCGAA AAAGAAG  
144 GAUGUAG CUGAUGAGGCCGAAAGGCCGAA AAAAGAA  
144 GAUGUAG CUGAUGAGGCCGAAAGGCCGAA AAAAGAA  
144 GAUGUAG CUGAUGAGGCCGAAAGGCCGAA AAAAGAA  
147 AGAGAUG CUGAUGAGGCCGAAAGGCCGAA AGAAAAA  
153 AGAAACA CUGAUGAGGCCGAAAGGCCGAA AGAUGUA  
165 UCACAAA CUGAUGAGGCCGAAAGGCCGAA AUCGAGA  
165 UCACAAA CUGAUGAGGCCGAAAGGCCGAA AUCGAGA  
165 UCACAAA CUGAUGAGGCCGAAAGGCCGAA AUCGAGA  
166 CUCACAA CUGAUGAGGCCGAAAGGCCGAA AAUCGAG  
167 GCUCACA CUGAUGAGGCCGAAAGGCCGAA AAAUCGA  
167 GCUCACA CUGAUGAGGCCGAAAGGCCGAA AAAUCGA  
167 GCUCACA CUGAUGAGGCCGAAAGGCCGAA AAAUCGA  
168 GGCUCAC CUGAUGAGGCCGAAAGGCCGAA AAAAUCG  
168 GGCUCAC CUGAUGAGGCCGAAAGGCCGAA AAAAUCG  
197 UAGAGCC CUGAUGAGGCCGAAAGGCCGAA AUGGAGC  
202 GAAUCTA CUGAUGAGGCCGAAAGGCCGAA AGCCAAU  
208 AGCCAGG CUGAUGAGGCCGAAAGGCCGAA AUCTAGA  
216 AUGGGGA CUGAUGAGGCCGAAAGGCCGAA AGCCAGG  
217 GAUGGGG CUGAUGAGGCCGAAAGGCCGAA AAGCCAG  
217 GAUGGGG CUGAUGAGGCCGAAAGGCCGAA AAGCCAG  
217 GAUGGGG CUGAUGAGGCCGAAAGGCCGAA AAGCCAG  
218 UGAUGGG CUGAUGAGGCCGAAAGGCCGAA AAAGCCA  
218 UGAUGGG CUGAUGAGGCCGAAAGGCCGAA AAAGCCA  
218 UGAUGGG CUGAUGAGGCCGAAAGGCCGAA AAAGCCA  
218 UGAUGGG CUGAUGAGGCCGAAAGGCCGAA AAAGCCA  
224 AGAACAU CUGAUGAGGCCGAAAGGCCGAA AUGGGGA  
224 AGAACAU CUGAUGAGGCCGAAAGGCCGAA AUGGGGA  
230 CUUUGGA CUGAUGAGGCCGAAAGGCCGAA AACADGA  
232 UGCUUUG CUGAUGAGGCCGAAAGGCCGAA AGAACAU  
232 UGCUUUG CUGAUGAGGCCGAAAGGCCGAA AGAACAU  
232 UGCUUUG CUGAUGAGGCCGAAAGGCCGAA AGAACAU  
241 AGCTUCA CUGAUGAGGCCGAAAGGCCGAA AUGCTUU  
241 AGCTUCA CUGAUGAGGCCGAAAGGCCGAA AUGCTUU  
241 AGCTUCA CUGAUGAGGCCGAAAGGCCGAA AUGCTUU  
249 CAAGCCA CUGAUGAGGCCGAAAGGCCGAA AGCTUCA  
264 AUCAACT CUGAUGAGGCCGAAAGGCCGAA ACAAUTG  
287 ACTUGAG CUGAUGAGGCCGAAAGGCCGAA AGUGGUG  
295 ACAUGGA CUGAUGAGGCCGAAAGGCCGAA ACTUGAG



295	ACAUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACUUGAG
296	GACAUGG	CUGAUGAGGCCGAAAGGCCGAA	AACUUGA
297	GGACAUG	CUGAUGAGGCCGAAAGGCCGAA	AAACUUG
297	GGACAUG	CUGAUGAGGCCGAAAGGCCGAA	AAACUUG
314	AGAGAAG	CUGAUGAGGCCGAAAGGCCGAA	AUGAGCC
314	AGAGAAG	CUGAUGAGGCCGAAAGGCCGAA	AUGAGCC
315	AAGAGAA	CUGAUGAGGCCGAAAGGCCGAA	AAUGAGC
315	AAGAGAA	CUGAUGAGGCCGAAAGGCCGAA	AAUGAGC
317	CAAAGAG	CUGAUGAGGCCGAAAGGCCGAA	AGAAUGA
318	ACAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGAAUG
318	ACAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGAAUG
320	GCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGAA
320	GCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGAA
322	CAGCACA	CUGAUGAGGCCGAAAGGCCGAA	AGAGAAG
322	CAGCACA	CUGAUGAGGCCGAAAGGCCGAA	AGAGAAG
323	GCAGCAC	CUGAUGAGGCCGAAAGGCCGAA	AAGAGAA
336	GAAAGAC	CUGAUGAGGCCGAAAGGCCGAA	AAUCAGC
341	CUUGUGA	CUGAUGAGGCCGAAAGGCCGAA	AGACGAA
341	CUUGUGA	CUGAUGAGGCCGAAAGGCCGAA	AGACGAA
342	ACTUGUG	CUGAUGAGGCCGAAAGGCCGAA	AAGACGA
343	CACUUGU	CUGAUGAGGCCGAAAGGCCGAA	AAAGACG
343	CACUUGU	CUGAUGAGGCCGAAAGGCCGAA	AAAGACG
352	AUCUGAA	CUGAUGAGGCCGAAAGGCCGAA	ACACUUG
355	AACAUCU	CUGAUGAGGCCGAAAGGCCGAA	AAGACAC
382	UUUCACU	CUGAUGAGGCCGAAAGGCCGAA	ACTUGGA
408	UAACGGC	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGC
414	GAGUUGU	CUGAUGAGGCCGAAAGGCCGAA	ACGCCAA
414	GAGUUGU	CUGAUGAGGCCGAAAGGCCGAA	ACGCCAA
421	AUGAGGA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGUA
426	UCUUCAU	CUGAUGAGGCCGAAAGGCCGAA	AGGAGAG
439	GUCUUCA	CUGAUGAGGCCGAAAGGCCGAA	ACTUCAUC
452	GCCAGUA	CUGAUGAGGCCGAAAGGCCGAA	AUUCGGU
454	UUGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAUUCG
484	AAUGACA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCAC
484	AAUGACA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCAC
488	CAGCAAU	CUGAUGAGGCCGAAAGGCCGAA	ACAGACA
503	ACACUUU	CUGAUGAGGCCGAAAGGCCGAA	AGUUUCC
503	ACACUUU	CUGAUGAGGCCGAAAGGCCGAA	AGUUUCC
520	GUUCUUA	CUGAUGAGGCCGAAAGGCCGAA	ACUCGGG
535	GUCAUAU	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCG
536	UGUCAUA	CUGAUGAGGCCGAAAGGCCGAA	AAAGUCC
538	GUUGUCA	CUGAUGAGGCCGAAAGGCCGAA	AUAAAGU
553	AAGAGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUAGU
553	AAGAGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUAGU
556	GAUAAGA	CUGAUGAGGCCGAAAGGCCGAA	AGUAGGU
556	GAUAAGA	CUGAUGAGGCCGAAAGGCCGAA	AGUAGGU
560	GGAUGAU	CUGAUGAGGCCGAAAGGCCGAA	AGAGAGU
561	AGGAUGA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGAG
561	AGGAUGA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGAG

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566	GGCCCAG	CUGAUGAGGCCCGAAAGGCCGAA	AUGAUAA
566	GGCCCAG	CUGAUGAGGCCCGAAAGGCCGAA	AUGAUAA
581	GGUCUGA	CUGAUGAGGCCCGAAAGGCCGAA	AGGACCA
583	CCGGUCU	CUGAUGAGGCCCGAAAGGCCGAA	AAAGGAC
583	CCGGUCU	CUGAUGAGGCCCGAAAGGCCGAA	AAAGGAC
598	ACAGCUG	CUGAUGAGGCCCGAAAGGCCGAA	AUGUGCC
608	UUUGAAC	CUGAUGAGGCCCGAAAGGCCGAA	ACACAGC
611	UCUUUUG	CUGAUGAGGCCCGAAAGGCCGAA	ACGACAC
611	UCUUUUG	CUGAUGAGGCCCGAAAGGCCGAA	ACGACAC
612	UUCUUUU	CUGAUGAGGCCCGAAAGGCCGAA	AACGACA
641	AGUGUUU	CUGAUGAGGCCCGAAAGGCCGAA	ACUUCAU
649	UAAAGCC	CUGAUGAGGCCCGAAAGGCCGAA	AGUGUUU
649	UAAAGCC	CUGAUGAGGCCCGAAAGGCCGAA	AGUGUUU
655	CUUUACU	CUGAUGAGGCCCGAAAGGCCGAA	AAGCCAA
656	ACUUUAC	CUGAUGAGGCCCGAAAGGCCGAA	AAAGCCA
659	ACAACTU	CUGAUGAGGCCCGAAAGGCCGAA	ACTAAAG
664	GAUGGAC	CUGAUGAGGCCCGAAAGGCCGAA	ACTUUAC
667	UUUGAUG	CUGAUGAGGCCCGAAAGGCCGAA	ACAACTU
671	CAGCTUU	CUGAUGAGGCCCGAAAGGCCGAA	AUGGACA
682	GGUAGAG	CUGAUGAGGCCCGAAAGGCCGAA	AGUCAGC
682	GGUAGAG	CUGAUGAGGCCCGAAAGGCCGAA	AGUCAGC
682	GGUAGAG	CUGAUGAGGCCCGAAAGGCCGAA	AGUCAGC
683	GGGUAGA	CUGAUGAGGCCCGAAAGGCCGAA	AAGUCAG
683	GGGUAGA	CUGAUGAGGCCCGAAAGGCCGAA	AAGUCAG
685	GGGGGUA	CUGAUGAGGCCCGAAAGGCCGAA	AGAAGUC
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687	UUUGGGG	CUGAUGAGGCCCGAAAGGCCGAA	AGAGAAG
698	ACUCAGU	CUGAUGAGGCCCGAAAGGCCGAA	AUGUUGG
698	ACUCAGU	CUGAUGAGGCCCGAAAGGCCGAA	AUGUUGG
718	GUCUGCA	CUGAUGAGGCCCGAAAGGCCGAA	AUGGGUU
718	GUCUGCA	CUGAUGAGGCCCGAAAGGCCGAA	AUGGGUU
729	AUCCUUU	CUGAUGAGGCCCGAAAGGCCGAA	AGUGUCU
729	AUCCUUU	CUGAUGAGGCCCGAAAGGCCGAA	AGUGUCU
729	AUCCUUU	CUGAUGAGGCCCGAAAGGCCGAA	AGUGUCU
737	AGCAGGU	CUGAUGAGGCCCGAAAGGCCGAA	AUCCUUU
737	AGCAGGU	CUGAUGAGGCCCGAAAGGCCGAA	AUCCUUU
737	AGCAGGU	CUGAUGAGGCCCGAAAGGCCGAA	AUCCUUU
745	GGAAGCA	CUGAUGAGGCCCGAAAGGCCGAA	AGCAGGU
745	GGAAGCA	CUGAUGAGGCCCGAAAGGCCGAA	AGCAGGU
759	UUUGGGA	CUGAUGAGGCCCGAAAGGCCGAA	ACCCCCG
759	UUUGGGA	CUGAUGAGGCCCGAAAGGCCGAA	ACCCCCG
759	UUUGGGA	CUGAUGAGGCCCGAAAGGCCGAA	ACCCCCG
760	CUUUGGG	CUGAUGAGGCCCGAAAGGCCGAA	AACCCCC
760	CUUUGGG	CUGAUGAGGCCCGAAAGGCCGAA	AACCCCC
760	CUUUGGG	CUGAUGAGGCCCGAAAGGCCGAA	AACCCCC
761	GCUUUGG	CUGAUGAGGCCCGAAAGGCCGAA	AAACCCC
771	GAGAAGC	CUGAUGAGGCCCGAAAGGCCGAA	AGGCUUU
771	GAGAAGC	CUGAUGAGGCCCGAAAGGCCGAA	AGGCUUU

776	ACCAAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGCGAG
776	ACCAAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGCGAG
778	CAACCAA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGCG
784	AUUUUC	CUGAUGAGGCCGAAAGGCCGAA	ACCAAGA
803	UGCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AAUUCUC
803	UGCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AAUUCUC
803	UGCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AAUUCUC
812	UCGUUU	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAG
812	UCGUUU	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAG
816	AUUGUCG	CUGAUGAGGCCGAAAGGCCGAA	AUUGAUG
816	AUUGUCG	CUGAUGAGGCCGAAAGGCCGAA	AUUGAUG
824	CCUGGGA	CUGAUGAGGCCGAAAGGCCGAA	AUUGUCG
825	UCCUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAUUGUC
826	AUCCUGG	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGU
834	GAUUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCCUGG
841	CAAUUCA	CUGAUGAGGCCGAAAGGCCGAA	AUUCAGG
841	CAAUUCA	CUGAUGAGGCCGAAAGGCCGAA	AUUCAGG
850	AAUGGUG	CUGAUGAGGCCGAAAGGCCGAA	ACAAUUC
869	UGAAAUC	CUGAUGAGGCCGAAAGGCCGAA	AGUUGGC
869	UGAAAUC	CUGAUGAGGCCGAAAGGCCGAA	AGUUGGC
869	UGAAAUC	CUGAUGAGGCCGAAAGGCCGAA	AGUUGGC
873	GUUUUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCUAGU
873	GUUUUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCUAGU
874	CGUUAUG	CUGAUGAGGCCGAAAGGCCGAA	AAUCUAG
875	UCGUUU	CUGAUGAGGCCGAAAGGCCGAA	AAAUUCU
885	UGGUUGC	CUGAUGAGGCCGAAAGGCCGAA	AGUCGUA
899	GACACUU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGU
899	GACACUU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGU
906	UUAAUGA	CUGAUGAGGCCGAAAGGCCGAA	ACACUUA
906	UUAAUGA	CUGAUGAGGCCGAAAGGCCGAA	ACACUUA
908	AUUUAAU	CUGAUGAGGCCGAAAGGCCGAA	AGACACU
911	CAUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AUGAGAC
916	AUCUCCA	CUGAUGAGGCCGAAAGGCCGAA	AUUUAAU
916	AUCUCCA	CUGAUGAGGCCGAAAGGCCGAA	AUUUAAU
943	CCAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AGUCCUC
944	CCCAGGU	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCU
1001	CUGCCCC	CUGAUGAGGCCGAAAGGCCGAA	AAGAGCA
1034	CGAUGAC	CUGAUGAGGCCGAAAGGCCGAA	ACGACUG
1037	CAACGAU	CUGAUGAGGCCGAAAGGCCGAA	ACGACGA
1043	UGAUGAC	CUGAUGAGGCCGAAAGGCCGAA	ACGAUGA
1046	UGAUGAU	CUGAUGAGGCCGAAAGGCCGAA	ACAACGA
1049	AUUUGAU	CUGAUGAGGCCGAAAGGCCGAA	AUGACAA
1060	CUUACAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAUUU
1060	CUUACAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAUUU
1060	CUUACAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAUUU
1060	CUUACAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAUUU
1061	GUUUACA	CUGAUGAGGCCGAAAGGCCGAA	AAGCAUU
1080	CUUCUGA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCTU
1080	CUUCUGA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCTU

1081 UCUUCUG CUGAUGAGGCCGAAAGGCCGAA AACAGCU  
1121 CGAAGGU CUGAUGAGGCCGAAAGGCCGAA AGGCUGU  
1121 CGAAGGU CUGAUGAGGCCGAAAGGCCGAA AGGCUGU  
1121 CGAAGGU CUGAUGAGGCCGAAAGGCCGAA AGGCUGU  
1122 CCGAAGG CUGAUGAGGCCGAAAGGCCGAA AAGGCUG  
1126 AGGCCCG CUGAUGAGGCCGAAAGGCCGAA AGGUAAG  
1127 CAGGCC CUGAUGAGGCCGAAAGGCCGAA AAGGUA  
1127 CAGGCC CUGAUGAGGCCGAAAGGCCGAA AAGGUA  
1144 UUCAGCU CUGAUGAGGCCGAAAGGCCGAA AUGCUUC  
1144 UUCAGCU CUGAUGAGGCCGAAAGGCCGAA AUGCUUC  
1145 GUUCAGC CUGAUGAGGCCGAAAGGCCGAA AAUGCTU  
1160 AAAGGAA CUGAUGAGGCCGAAAGGCCGAA ACGGUCTU  
1162 CUAAGG CUGAUGAGGCCGAAAGGCCGAA AGACGGU  
1163 ACUAAG CUGAUGAGGCCGAAAGGCCGAA AAGACGG  
1167 AAGAACTU CUGAUGAGGCCGAAAGGCCGAA AAGGAAG  
1177 AUGGACA CUGAUGAGGCCGAAAGGCCGAA AGAAGAA  
1181 CCACAUG CUGAUGAGGCCGAAAGGCCGAA ACAGAGA  
1181 CCACAUG CUGAUGAGGCCGAAAGGCCGAA ACAGAGA  
1192 UACCAUG CUGAUGAGGCCGAAAGGCCGAA AUCCAC  
1199 CACAUA CUGAUGAGGCCGAAAGGCCGAA ACCAUGU  
1201 GCCACAU CUGAUGAGGCCGAAAGGCCGAA AUACCAU  
1210 ACCUCAU CUGAUGAGGCCGAAAGGCCGAA AGCCACA  
1210 ACCUCAU CUGAUGAGGCCGAAAGGCCGAA AGCCACA  
1223 AAAGAAA CUGAUGAGGCCGAAAGGCCGAA AUUGUAC  
1225 UGAAAGA CUGAUGAGGCCGAAAGGCCGAA AGAUUGU  
1225 UGAAAGA CUGAUGAGGCCGAAAGGCCGAA AGAUUTU  
1226 CUGAAAG CUGAUGAGGCCGAAAGGCCGAA AAGA  
1227 GCUGAAA CUGAUGAGGCCGAAAGGCCGAA AAAGUU  
1227 GCUGAAA CUGAUGAGGCCGAAAGGCCGAA AAAGAUU  
1227 GCUGAAA CUGAUGAGGCCGAAAGGCCGAA AAAGAUU  
1229 GUGCUGA CUGAUGAGGCCGAAAGGCCGAA AGAAAGA  
1230 GGUCUG CUGAUGAGGCCGAAAGGCCGAA AAGAAAG  
1252 UGUCCGA CUGAUGAGGCCGAAAGGCCGAA AGAUCAG  
1274 UUAACUC CUGAUGAGGCCGAAAGGCCGAA AUCUUGU  
1310 GGAAAGA CUGAUGAGGCCGAAAGGCCGAA AUCCUCA  
1312 AUGGAAA CUGAUGAGGCCGAAAGGCCGAA AAUUCTU  
1314 UGAUGGA CUGAUGAGGCCGAAAGGCCGAA AGAAUUC  
1316 CTUGAUG CUGAUGAGGCCGAAAGGCCGAA AAAGAAA  
1320 GCUUCCU CUGAUGAGGCCGAAAGGCCGAA AUGGAAA  
1320 GCUUCCU CUGAUGAGGCCGAAAGGCCGAA AUGGAAA  
1339 CCCAGCA CUGAUGAGGCCGAAAGGCCGAA ACTUGCC  
1355 AUCAAGC CUGAUGAGGCCGAAAGGCCGAA AUCAAAG  
1437 UUUUUCU CUGAUGAGGCCGAAAGGCCGAA AUACCAC  
1437 UUUUUCU CUGAUGAGGCCGAAAGGCCGAA AUACCAC  
1475 GCAGUA CUGAUGAGGCCGAAAGGCCGAA ACUAGGC  
1477 UUGCAGU CUGAUGAGGCCGAAAGGCCGAA AGACTAG  
1487 ACAUAUC CUGAUGAGGCCGAAAGGCCGAA AGUUGCA  
1491 CAUGACA CUGAUGAGGCCGAAAGGCCGAA AUCAAGU  
1491 CAUGACA CUGAUGAGGCCGAAAGGCCGAA AUCAAGU

1505	AGACACC	CUGAUGAGGCCGAAAGGCCGAA	ACCAAAC
1530	CUUCAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGGGCA
1531	UCUUCAG	CUGAUGAGGCCGAAAGGCCGAA	AAAGGGC
1532	CUUUUCA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGGG
1532	CUUUUCA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGGG
1644	ACAUCCC	CUGAUGAGGCCGAAAGGCCGAA	ACCAUAG
1652	CCGUUUU	CUGAUGAGGCCGAAAGGCCGAA	ACAUCCC
1652	CCGUUUU	CUGAUGAGGCCGAAAGGCCGAA	ACAUCCC
1670	UAAUAUU	CUGAUGAGGCCGAAAGGCCGAA	AUAUUAU
1674	UAUUUAA	CUGAUGAGGCCGAAAGGCCGAA	AUUUAUA
1676	UUUAUUU	CUGAUGAGGCCGAAAGGCCGAA	AUAUUUA
1677	UUUUUAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUU
1677	UUUUUAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUU
1694	UUUGCUC	CUGAUGAGGCCGAAAGGCCGAA	AUACUCU

Tabl BVI: Human B7-2 Hammerhead Ribozyme Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
16	GAAAGCU U UGCUUCU	271	UAGUAGU A UUUUGGC
17	AAAGCUU U GCUUCUC	273	GUAGUUAU U UUGGCAG
21	CUUUGCU U CUCUGCU	274	UAGUUAU U UGGCAGG
22	UUUGCUU C UCUGCUG	275	AGUUAUU U GGCAGGA
24	UGCUUCU C UGUGCUU	294	GAAAACU U GGUUCUG
34	CUGCUGU A ACAGGGA	298	ACUUGGU U CUGAAUG
44	AGGGACU A GCACAGA	299	CUUGGUU C UGAAUGA
70	GUGGGGU C AUUCCA	310	AUGAGGU A UACUAG
73	GGGUCAU U UCCAGAU	312	GAGGUUAU A CUUAGGC
74	GGUCAU U CCAGAU	315	GUUAUACU U AGGCAAA
75	GUCAUUU C CAGAUU	316	UAUACUU A GGCAAG
81	UCCAGAU A UUAGGUC	330	GAGAAU U UGACAGU
83	CAGAUU A AGGUCAC	331	AGAAAUU U GACAGUG
84	AGAUUU A GGUCACA	340	ACAGUGU U CAUCCA
88	AUUAGGU C ACAGCAG	341	CAGUGU C AUUCCAA
113	AAUGGAU C CCCAGUG	344	UGUUCAU U CCAAGUA
125	GUGCACU A UGGGACU	345	GUUCAU C CAAGUAU
137	ACUGAGU A ACAUUCU	351	UCCAAGU A UAUGGCG
142	GUACAU U CUCUUUG	353	CAAGUAU A UGGGCG
143	UAACAU C UCUUUGU	368	CACAAGU U UUGGCG
145	ACAUUCU C UUUGUGA	369	ACAAGUU U UGAUUGG
147	AUUCUU U UGUGAUG	370	CAAGUUU U GAUUGG
148	UUCUUU U GUGAUGG	374	UUUUGAU U CGGACAG
159	AUGGCCU U CCUGCUC	375	UUUGAUU C GGACAGU
160	UGGCUU C CUGCUCU	383	GGACAGU U GGACCCU
166	UCCUGCU C UCUGGUG	397	UGAGACU U CACAAUC
168	CUGCUCU C UGGUGCU	398	GAGACUU C ACAAUUCU
179	UGCUGCU C CUCUGAA	404	UCACAAU C UUCAGAU
182	UGCUCU C UGAAGAU	406	ACAAUCU U CAGAUCA
190	UGAAGAU U CAAGCUU	407	CAAUCUU C AGAUCAA
191	GAAGAUU C AAGCUUA	412	UUCAGAU C AAGGACA
197	UCAAGCU U AUUCAA	426	AAGGGCU U GUAUCAA
198	CAAGCUU A UUUCAU	429	GGCUUGU A UCAAUGU
200	AGCUUAU U UCAAUGA	431	CUUGUAU C AAUGUAU
201	GCUUAUU U CAAUGAG	437	UCAUUGU A UCAUCCA
202	CUUAUUU C AAUGAGA	439	AAUGUAU C AUCCAUC
231	UGCCAAU U UGCAAAAC	442	GUUAUUAU C CAUCACA
232	GCCAAU U GCAAAACU	446	CAUCCAU C ACAAAAA
240	GCAAACU C UCAAAAC	469	GAAUGAU U CGCAUCC
242	AAACUCU C AAAACCA	470	AAUGAUU C GCAUCCA
265	GUGAGCU A GUAGUAU	475	UUCGCAU C CACCAGA
268	AGCUAGU A GUUUUU	488	GAUGAAU U UGAACU

489	AUGAAUU C UGAACUG	721	UGUCUGU U UCAUUCU
498	GAACUGU C AGUGCUU	722	GUCUGUU U CAUUCUU
505	CAGUGCU U GCUAACU	723	UCUGUUU C AUUCCUU
509	GCUUGCU A ACUUCAG	726	GUUUCAU U CCCUGAU
513	GCUAACU U CAGUCAA	727	UUUCAUU C CCUGAUG
514	CUAACUU C AGUCAAC	736	CUGAUGU U ACGAGCA
518	CUUCAGU C AACCUGA	737	UGAUGUU A CGAGCAA
529	CUGAAAU A GUACCAA	746	GAGCAAU A UGACCAU
532	AAAUAGU A CCAAUUU	754	UGACCAU C UUCUGUA
538	UACCAAU U UCUAUAU	756	ACCAUCU U CUGUAUU
539	ACCAAUU U CUAUAUU	757	CCAUCUU C UGUUUCU
540	CCAUAUU C UUAUAUA	761	CUUCUGU A UUCUGGA
542	AAUUCUU A AUAUAAC	763	UCUGUAU U CUGGAAA
545	UUCUAUU A UAACAGA	764	CUGUAUU C UGGAAAC
547	CUAUAUU A ACAGAAA	787	CGCGGCU U UUAUCUU
561	AAUGUGU A CAUAAAU	788	GCGGCUU U UAUUCUC
565	UGUACAU A AAUUGA	789	CGGCUUU U AUCUUCU
569	CAUAAAU U UGACCUU	790	GGCUUUU A UCUUCAC
570	AUAUAUU U GACCUUC	792	CUUUUAU C UUCACCU
579	ACCUGCU C AUCUAUA	794	UUUAUCU U CACCUUU
582	UGCUCAU C UAUAAC	795	UUAUCUU C ACCUUUC
584	CUCAUCU A UAACCGG	800	UUCACCU U UCUUAU
586	CAUCUAU A CACGGUU	801	UCACCUU U CUUAUA
593	ACACGGU U ACCCAGA	802	CACCUUU C UCUAUA
594	CACGGUU A CCCAGAA	804	CCUUUCU C UUAAGAG
605	AGAACCU A AGAAGAU	806	UUUCUCU A UAGAGCU
619	UGAGUGU U UUGCUAA	808	UCUCUAU A GAGCUUG
620	GAGUGUU U UGCUAAG	814	UAGAGCU U GAGGACC
621	AGUGUUU U GCUAAGA	824	GGACCCU C AGCCUCC
625	UUUUGCU A AGAACCA	830	UCAGCCU C CCCCAGA
638	CAAGAAU U CAACUAU	844	ACCACAU U CCUUGGA
639	AAGAAUU C AACUAUC	845	CCACAUU C CUUGGAU
644	UUCAACU A UCGAGUA	848	CAUUCUU U GGAUUAC
646	CAACUAU C GAGUAUG	853	CUUGGAU U ACAGCUG
651	AUCGAGU A UGAUGGU	854	UUGGAUU A CAGCUGU
659	UGAUGGU A UUAUGCA	862	CAGCUGU A CUUCCAA
661	AUGGUUU U AUGCAGA	865	CUGUACU U CCAACAG
662	UGGUUUU A UGCAGAA	866	UGUACUU C CAACAGU
672	CAGAAAU C UCAAGAU	874	CAACAGU U AUUAUAU
674	GAAAUUU C AAGAUAA	875	AACAGUU A UUAUAUG
680	UCAAGAU A AUGUCAC	877	CAGUUAU U AUAUGUG
685	AUAUAUU C ACAGAAC	878	AGUUAUU A UAUGUGU
696	GAACUGU A CGACGUU	880	UUAUAUU A UGUGUGA
703	ACGACGU U UCCAUCA	892	UGAUGGU U UUCUGUC
704	CGACGUU U CCAUCAG	893	G AUGGUU U UCUGUCU
705	GACGUUU C CAUCAGC	894	AUGGUUU U CUGUCUA
709	UUUCCAU C AGCUUGU	895	UUGUUUU C UGUCUAA
714	AUCAGCU U GUCUGUU	899	UUUCUGU C UAAUUCU
717	AGCUUGU C UGUUUCA	901	UCUGUCU A AUUCUAU

Table BVII: Human B7-2 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequences
16	AGAAGCA CUGAUGAGGCCGAAAGGCCGAA AGCUUUC
17	GAGAAGC CUGAUGAGGCCGAAAGGCCGAA AAGCUUU
21	AGCAGAG CUGAUGAGGCCGAAAGGCCGAA AGCAAAG
22	CAGCAGA CUGAUGAGGCCGAAAGGCCGAA AAGCAAA
24	AGCAGCA CUGAUGAGGCCGAAAGGCCGAA AGAAGCA
34	UCCUGU CUGAUGAGGCCGAAAGGCCGAA ACAGCAG
44	UCUGUGC CUGAUGAGGCCGAAAGGCCGAA AGUCCCU
70	UGGAAAU CUGAUGAGGCCGAAAGGCCGAA ACCCCAC
73	AUCUGGA CUGAUGAGGCCGAAAGGCCGAA AUGACCC
74	UAUCUGG CUGAUGAGGCCGAAAGGCCGAA AAUGACC
75	AUAUCUG CUGAUGAGGCCGAAAGGCCGAA AAUAGAC
81	GACCUAA CUGAUGAGGCCGAAAGGCCGAA AUCUGGA
83	GUGACCU CUGAUGAGGCCGAAAGGCCGAA AUUAUCU
84	UGUGACC CUGAUGAGGCCGAAAGGCCGAA AAUAUCU
88	CUGCUGU CUGAUGAGGCCGAAAGGCCGAA ACCUAAU
113	CACUGGG CUGAUGAGGCCGAAAGGCCGAA AUCCAUU
125	AGUCCCA CUGAUGAGGCCGAAAGGCCGAA AGUGCAC
137	AGAAUGU CUGAUGAGGCCGAAAGGCCGAA ACUCAGU
142	CAAAGAG CUGAUGAGGCCGAAAGGCCGAA AUGUUAC
143	ACAAAGA CUGAUGAGGCCGAAAGGCCGAA AAUGUUA
145	UCACAAA CUGAUGAGGCCGAAAGGCCGAA AGAAUGU
147	CAUCACA CUGAUGAGGCCGAAAGGCCGAA AGAGAAU
148	CCAUCAC CUGAUGAGGCCGAAAGGCCGAA AAGAGAA
159	GAGCAGG CUGAUGAGGCCGAAAGGCCGAA AGGCCAU
160	AGAGCAG CUGAUGAGGCCGAAAGGCCGAA AAGGCCA
166	CACCAGA CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
168	AGCACCA CUGAUGAGGCCGAAAGGCCGAA AGAGCAG
179	UUCAGAG CUGAUGAGGCCGAAAGGCCGAA AGCAGCA
182	AUCUUCA CUGAUGAGGCCGAAAGGCCGAA AGGAGCA
190	AAGCUUG CUGAUGAGGCCGAAAGGCCGAA AUCUUCA
191	UAAGCUU CUGAUGAGGCCGAAAGGCCGAA AAUCUUC
197	UUGAAAU CUGAUGAGGCCGAAAGGCCGAA AGCUUGA
198	AUUGAAA CUGAUGAGGCCGAAAGGCCGAA AAGCUUG
200	UCAUUGA CUGAUGAGGCCGAAAGGCCGAA AUAAGCU
201	CUCAUUG CUGAUGAGGCCGAAAGGCCGAA AAUAAGC
202	UCUCAUU CUGAUGAGGCCGAAAGGCCGAA AAUAAG
231	GUUUGCA CUGAUGAGGCCGAAAGGCCGAA AUUGGCA
232	AGUUUGC CUGAUGAGGCCGAAAGGCCGAA AAUUGGC
240	GUUUUGA CUGAUGAGGCCGAAAGGCCGAA AGUUUGC
242	UGGUUUU CUGAUGAGGCCGAAAGGCCGAA AGAGUUU
265	AUACUAC CUGAUGAGGCCGAAAGGCCGAA AGCUCAC



268	AAAAUAC	CUGAUGAGGCCGAAAGGCCGAA	ACUAGCU
271	GCCAAA	CUGAUGAGGCCGAAAGGCCGAA	ACUACUA
273	CUGCCAA	CUGAUGAGGCCGAAAGGCCGAA	AUACUAC
274	CCUGCCA	CUGAUGAGGCCGAAAGGCCGAA	AAUACUA
275	UCCUGCC	CUGAUGAGGCCGAAAGGCCGAA	AAAUACU
294	CAGAAC	CUGAUGAGGCCGAAAGGCCGAA	AGUUUUC
298	CAUUCAG	CUGAUGAGGCCGAAAGGCCGAA	ACCAAGU
299	UCAUUA	CUGAUGAGGCCGAAAGGCCGAA	AACCAAG
310	CUAAGUA	CUGAUGAGGCCGAAAGGCCGAA	ACCUCAU
312	GCCUAAG	CUGAUGAGGCCGAAAGGCCGAA	AUACUUC
315	UUUGCCU	CUGAUGAGGCCGAAAGGCCGAA	AGUAUAC
316	CUUUGCC	CUGAUGAGGCCGAAAGGCCGAA	AAGUAUA
330	ACUGUCA	CUGAUGAGGCCGAAAGGCCGAA	AUUUCUC
331	CACUGUC	CUGAUGAGGCCGAAAGGCCGAA	AAUUUCU
340	UGGAAUG	CUGAUGAGGCCGAAAGGCCGAA	ACACUGU
341	UUGGAAU	CUGAUGAGGCCGAAAGGCCGAA	AACACUG
344	UACUUGG	CUGAUGAGGCCGAAAGGCCGAA	AUGAACA
345	AUACUUG	CUGAUGAGGCCGAAAGGCCGAA	AAUGAAC
351	GCCCAUA	CUGAUGAGGCCGAAAGGCCGAA	ACUUGGA
353	CGGCCCA	CUGAUGAGGCCGAAAGGCCGAA	AUACUUG
368	GAAUCAA	CUGAUGAGGCCGAAAGGCCGAA	ACUUGUG
369	CGAAUCA	CUGAUGAGGCCGAAAGGCCGAA	AACTUGU
370	CCGAAUC	CUGAUGAGGCCGAAAGGCCGAA	AAACUUG
374	CUGUCCG	CUGAUGAGGCCGAAAGGCCGAA	AUCAAAA
375	ACUGUCC	CUGAUGAGGCCGAAAGGCCGAA	AAUCAAA
383	AGGGUCC	CUGAUGAGGCCGAAAGGCCGAA	ACTUGCC
397	GAUUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGUUGCA
398	AGAUUGU	CUGAUGAGGCCGAAAGGCCGAA	AAUUCUC
404	AUCUGAA	CUGAUGAGGCCGAAAGGCCGAA	AUUGUGA
406	UGAUCUG	CUGAUGAGGCCGAAAGGCCGAA	AGAUUGU
407	UUGAUCU	CUGAUGAGGCCGAAAGGCCGAA	AAGAUUG
412	UGUCCUU	CUGAUGAGGCCGAAAGGCCGAA	AUCUGAA
426	UUGAUAC	CUGAUGAGGCCGAAAGGCCGAA	AGCCCUU
429	ACAUUGA	CUGAUGAGGCCGAAAGGCCGAA	ACAAGCC
431	AUACAUU	CUGAUGAGGCCGAAAGGCCGAA	AUACAAG
437	UGGAUGA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUGA
439	GAUGGAU	CUGAUGAGGCCGAAAGGCCGAA	AUACAUU
442	UGUGAUG	CUGAUGAGGCCGAAAGGCCGAA	AUGAUAC
446	UUUUUGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGAUG
469	GGAUGCG	CUGAUGAGGCCGAAAGGCCGAA	AUCAUUC
470	UGGAUGC	CUGAUGAGGCCGAAAGGCCGAA	AAUCAUU
475	UCUGGUG	CUGAUGAGGCCGAAAGGCCGAA	AUGCGAA
488	AGUUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUUCALC
489	CAGUUA	CUGAUGAGGCCGAAAGGCCGAA	AAUUCAU
498	AAGCACU	CUGAUGAGGCCGAAAGGCCGAA	ACAGUUC
505	AGUUAGC	CUGAUGAGGCCGAAAGGCCGAA	AGCACTUG
509	CUGAAGU	CUGAUGAGGCCGAAAGGCCGAA	AGCAAGC
513	UUGACUG	CUGAUGAGGCCGAAAGGCCGAA	AGUUAGC
514	GUUGACU	CUGAUGAGGCCGAAAGGCCGAA	AAGUUAG

518 UCAGGUU CUGAUGAGGCCGAAAGGCCGAA ACUGAAG  
529 UUGGUAC CUGAUGAGGCCGAAAGGCCGAA AUUUCAG  
532 AAAUUGG CUGAUGAGGCCGAAAGGCCGAA ACUAUUU  
538 UAUAAGA CUGAUGAGGCCGAAAGGCCGAA AUUGGUA  
539 AUAUUAG CUGAUGAGGCCGAAAGGCCGAA AAUUGGU  
540 UAUAUUA CUGAUGAGGCCGAAAGGCCGAA AAAUUGG  
542 GUUAUAU CUGAUGAGGCCGAAAGGCCGAA AGAAAUU  
545 UCUGUUA CUGAUGAGGCCGAAAGGCCGAA AUUAGAA  
547 UUUCUGU CUGAUGAGGCCGAAAGGCCGAA AUAUUAG  
561 AUUUUAG CUGAUGAGGCCGAAAGGCCGAA ACACAUU  
565 UCAAAUU CUGAUGAGGCCGAAAGGCCGAA AUGUACA  
569 CAGGUCA CUGAUGAGGCCGAAAGGCCGAA AUUUUAG  
570 GCAGGUC CUGAUGAGGCCGAAAGGCCGAA AAUUUUU  
579 UAUAAGU CUGAUGAGGCCGAAAGGCCGAA AGCAGGU  
582 GUGUAUA CUGAUGAGGCCGAAAGGCCGAA AUGAGCA  
584 CCGUGUA CUGAUGAGGCCGAAAGGCCGAA AGAUGAG  
586 AACCGUG CUGAUGAGGCCGAAAGGCCGAA AUAGAUG  
593 UCUGGGU CUGAUGAGGCCGAAAGGCCGAA ACCGUGU  
594 UUCUGGG CUGAUGAGGCCGAAAGGCCGAA AACCGUG  
605 AUCUUCU CUGAUGAGGCCGAAAGGCCGAA AGGUUCU  
619 UUAGCAA CUGAUGAGGCCGAAAGGCCGAA ACACUCA  
620 CUUAGCA CUGAUGAGGCCGAAAGGCCGAA AACACUC  
621 UCUUAGC CUGAUGAGGCCGAAAGGCCGAA AAACACU  
625 UGGUUCU CUGAUGAGGCCGAAAGGCCGAA AGCAAAA  
638 AUAGUUG CUGAUGAGGCCGAAAGGCCGAA AUUCUUG  
639 GAUAGUU CUGAUGAGGCCGAAAGGCCGAA AAUUCUU  
644 UACUCGA CUGAUGAGGCCGAAAGGCCGAA AGUUGAA  
646 CAUACUC CUGAUGAGGCCGAAAGGCCGAA AUAGUUG  
651 ACCAUCA CUGAUGAGGCCGAAAGGCCGAA ACUCGAU  
659 UGCAUAA CUGAUGAGGCCGAAAGGCCGAA ACCAUCA  
661 UCUGCAU CUGAUGAGGCCGAAAGGCCGAA AUACCAU  
662 UUCUGCA CUGAUGAGGCCGAAAGGCCGAA AAUACCA  
672 AUCUUGA CUGAUGAGGCCGAAAGGCCGAA AUUUCUG  
674 UUAUCUU CUGAUGAGGCCGAAAGGCCGAA AGAUUUC  
680 GUGACAU CUGAUGAGGCCGAAAGGCCGAA AUCUUGA  
685 GUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUUA  
696 AACGUUG CUGAUGAGGCCGAAAGGCCGAA ACAGUUC  
703 UGAUGGA CUGAUGAGGCCGAAAGGCCGAA ACGUUGU  
704 CUGAUGG CUGAUGAGGCCGAAAGGCCGAA AACGUUG  
705 GCUGAUG CUGAUGAGGCCGAAAGGCCGAA AAACGUC  
709 ACAAGCU CUGAUGAGGCCGAAAGGCCGAA AUGGAAA  
714 AACAGAC CUGAUGAGGCCGAAAGGCCGAA AGCUGAU  
717 UGAAACA CUGAUGAGGCCGAAAGGCCGAA ACAAGCU  
721 GGAAUGA CUGAUGAGGCCGAAAGGCCGAA ACAGACA  
722 GGGAAUG CUGAUGAGGCCGAAAGGCCGAA AACAGAC  
723 AGGGAAU CUGAUGAGGCCGAAAGGCCGAA AAACAGA  
726 AUCAGGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAC  
727 CAUCAGG CUGAUGAGGCCGAAAGGCCGAA AAUGAAA  
736 UGUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUCAG

737 UUGCUCG CUGAUGAGGCCGAAAGGCCGAA AACAUCA  
746 AUGGUCA CUGAUGAGGCCGAAAGGCCGAA AUUGCUC  
754 UACAGAA CUGAUGAGGCCGAAAGGCCGAA AUGGUCA  
756 AAUACAG CUGAUGAGGCCGAAAGGCCGAA AGAUGGU  
757 GAAUACA CUGAUGAGGCCGAAAGGCCGAA AAGAUGG  
761 UCCAGAA CUGAUGAGGCCGAAAGGCCGAA ACAGAAG  
763 UUUCCAG CUGAUGAGGCCGAAAGGCCGAA AUACAGA  
764 GUUUGCA CUGAUGAGGCCGAAAGGCCGAA AAUACAG  
787 AAGAUA CUGAUGAGGCCGAAAGGCCGAA AGCCGCG  
788 GAAGUA CUGAUGAGGCCGAAAGGCCGAA AAGCCGC  
789 UGAAGAU CUGAUGAGGCCGAAAGGCCGAA AAAGCCG  
790 GUGAAGA CUGAUGAGGCCGAAAGGCCGAA AAAAGCC  
792 AGGUGAA CUGAUGAGGCCGAAAGGCCGAA AUAAAAG  
794 AAAGGUG CUGAUGAGGCCGAAAGGCCGAA AGAUAAA  
795 GAAAGGU CUGAUGAGGCCGAAAGGCCGAA AAGAUAA  
800 AUAGAGA CUGAUGAGGCCGAAAGGCCGAA AGGUGAA  
801 UAUAGAG CUGAUGAGGCCGAAAGGCCGAA AAGGUGA  
802 CUAUAGA CUGAUGAGGCCGAAAGGCCGAA AAAGGUG  
804 CUCUAUA CUGAUGAGGCCGAAAGGCCGAA AGAAAGG  
806 AGCUCUA CUGAUGAGGCCGAAAGGCCGAA AGAGAAA  
808 CAAGCUC CUGAUGAGGCCGAAAGGCCGAA AUAGAGA  
814 GGUCCUC CUGAUGAGGCCGAAAGGCCGAA AGCUCUA  
824 GGAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGGUCC  
830 UCUGGGG CUGAUGAGGCCGAAAGGCCGAA AGGCUGA  
844 UCCAAGG CUGAUGAGGCCGAAAGGCCGAA AUGUGGU  
845 AUCCAAG CUGAUGAGGCCGAAAGGCCGAA AAUGUGG  
848 GUAAUCC CUGAUGAGGCCGAAAGGCCGAA AGGAAUG  
853 CAGCUGU CUGAUGAGGCCGAAAGGCCGAA AUCCAAG  
854 ACAGCUG CUGAUGAGGCCGAAAGGCCGAA AAUCCAA  
862 UUGGAAG CUGAUGAGGCCGAAAGGCCGAA ACAGCUG  
865 CUGUUGG CUGAUGAGGCCGAAAGGCCGAA AGUACAG  
866 ACUGUUG CUGAUGAGGCCGAAAGGCCGAA AAGUACA  
874 AUUAUAA CUGAUGAGGCCGAAAGGCCGAA ACUGUUG  
875 CAUAUAA CUGAUGAGGCCGAAAGGCCGAA AACUGUU  
877 CACAUAU CUGAUGAGGCCGAAAGGCCGAA AUAACUG  
878 ACACAU CUGAUGAGGCCGAAAGGCCGAA AAUAACU  
880 UCACACA CUGAUGAGGCCGAAAGGCCGAA AUAAUAA  
892 GACAGAA CUGAUGAGGCCGAAAGGCCGAA ACCAUCA  
893 AGACAGA CUGAUGAGGCCGAAAGGCCGAA AACCACU  
894 UAGACAG CUGAUGAGGCCGAAAGGCCGAA AAACCAU  
895 UUAGACA CUGAUGAGGCCGAAAGGCCGAA AAAACCA  
899 AGAAUUA CUGAUGAGGCCGAAAGGCCGAA ACAGAAA  
901 AUAGAAU CUGAUGAGGCCGAAAGGCCGAA AGACAGA  
904 UCCAUAG CUGAUGAGGCCGAAAGGCCGAA AUUAGAC  
905 UCCAUA CUGAUGAGGCCGAAAGGCCGAA AAUAGA  
907 AUUUGCA CUGAUGAGGCCGAAAGGCCGAA AGAAUUA  
935 GAGUUGC CUGAUGAGGCCGAAAGGCCGAA AGGCCGC  
942 UUUUAAA CUGAUGAGGCCGAAAGGCCGAA AGUUGCG  
944 CAUUUUU CUGAUGAGGCCGAAAGGCCGAA AGAGUUG

945	ACAUUUA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGUU
947	CCACAUU	CUGAUGAGGCCGAAAGGCCGAA	AUAAGAG
1009	GUUAUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUUUUU
1013	UCAGGUA	CUGAUGAGGCCGAAAGGCCGAA	AUGGAUU
1015	UUUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUAUGGA
1026	UUCAUCA	CUGAUGAGGCCGAAAGGCCGAA	AUCTUUC
1045	UUUUAUA	CUGAUGAGGCCGAAAGGCCGAA	ACACGCU
1046	CUUUUAA	CUGAUGAGGCCGAAAGGCCGAA	AACACGC
1047	ACUUUUA	CUGAUGAGGCCGAAAGGCCGAA	AAACACG
1048	AACUUUU	CUGAUGAGGCCGAAAGGCCGAA	AAAACAC
1049	GAACUUU	CUGAUGAGGCCGAAAGGCCGAA	AAAAACA
1055	GUCUUCG	CUGAUGAGGCCGAAAGGCCGAA	ACUUUUA
1056	UGUCUUC	CUGAUGAGGCCGAAAGGCCGAA	AACUUUU
1065	GCAUGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGUCUU
1067	UCGCAUG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGUC
1068	GUCGCAU	CUGAUGAGGCCGAAAGGCCGAA	AAGAUGU
1085	AAACAUG	CUGAUGAGGCCGAAAGGCCGAA	AUCACUU
1091	AAUUAUA	CUGAUGAGGCCGAAAGGCCGAA	ACAUGUA
1092	UAAUUAU	CUGAUGAGGCCGAAAGGCCGAA	AACAUGU
1093	UUAAUUA	CUGAUGAGGCCGAAAGGCCGAA	AAACAUG
1094	UUUAAUU	CUGAUGAGGCCGAAAGGCCGAA	AAAACAU
1095	CUUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AAAAACA
1098	ACUCUUU	CUGAUGAGGCCGAAAGGCCGAA	AUUAAAA
1099	UACUCUU	CUGAUGAGGCCGAAAGGCCGAA	AAUUAUA

Table BVIII: Mouse B7-2 Hammerhead Ribozyme Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
47	AcGGACU u GaACAac	194	cuUAuUU C aAUGGgA
47	aCggACU u gaAcAAC	208	acUGCaU a UCUGCcG
66	CUccUgU a gAcGUgU	210	UGCaUaU C UGCcGug
66	CUCcUgU A gAcGUgu	223	UGCCcAU U UaCAAAg
74	gAcGUgU u CcagAAC	223	UGCcCAU u UAcAaAg
83	CaGaACU U aCggaAG	224	GCCcAUU U aCBAAgg
134	caAuCcU U aUCUUUG	225	ccCAUUU a CAaAggc
134	CaauccU U AUCUUug	225	CccaUUU a cAAAgGc
134	caAUCcU u AuCUUug	242	AAaACAU a agCcUGa
134	CAaUccU U AUcUuUG	260	AGCUgGU A GUUUUUU
134	CAAUcCU U AUcUUUG	260	aGCUgGU a gUAUuUU
135	aAuCcUU a UCUUUGU	263	UgGUAGU A UUUUGGC
135	aAuCcUU a UCuuUgu	263	UGgUaGU a UUUUGgC
135	AaUccUU A UcUuUGU	265	GUAGUaU U UUGGCAG
135	aAUccUU a UCuuUgU	265	guAGUaU u UuGGCaG
137	uCcUUaU C UUUGUGA	266	UAGUaUU U UGGCAGG
137	UccUUaU c UuUGUGA	266	uAGUaUU U UGgcAgG
137	UCCuUAU c uuUGugA	266	UAgUauU u UGGcAgg
139	cUUAUCU U UGUGAca	267	AGUaUUU U GGCAGGA
140	UUaUCUU U GUGAcaG	267	AGUaUUU U GgcAgGA
140	UUaUcuU U guGACAG	286	cAAAaGU U GGUUCUG
149	UGAcaGU c UUGCUgA	286	CAAaagU U GgUUCuG
151	AcAGucU U GCUgaUC	290	AgUUUGU U CUGuAcG
151	AcaGuCU U gCUGaUC	291	gUUUGGU C UGuAcGA
158	UgCUgAU c UcAGaUg	295	GUUCuGU a CgAGcAc
158	UgCUgAU C UCaGaUG	304	GAGcAcU A uUUgGGC
158	UGcUgAU c uCagaUg	307	cacUAUU u GGgCACA
158	UgCUgAU c UCagaUg	323	AGAAAcU U GAuAGUG
160	CUGaUCU C aGaUGCU	343	gCCAAGU A ccUGGGC
160	cUGaUcU c AgAuGcU	343	gCCAagU a CCUgGGc
170	AUGcuGU u UcCgUgG	361	ACgAGcU U UGAcagG
171	UGCUGuU u CcgUGgA	381	cUGgACU c UacGACU
172	gCUgUuU C cgUgGAG	383	GgACUCU A CGACuUc
189	GcaaGcU u AUUUCaA	383	GGACuCU a cGaCUuC
189	gCAAGCU U AUUUCAA	389	uAcGacU u CaCAaUG
189	GCaaGCU u AuUUCaA	389	UacGACU U CACAAUG
190	CAAGCTU A UUUCAAU	390	acGACUC C ACAAUgU
190	CaAgcUU a uUUcaAU	390	ACgACUU c acAAUgU
192	AGCUUAU U UCAAUGg	398	ACAaUGU U CagauCA
192	aGCUUAU u UCAAUGg	398	ACAAUGU U CAGAUCA
193	GCUUAUU U CAAUGgG	398	ACaAuGU U cagAUCA
193	GcuUAuU U CaAUGGg	399	CAaUGUU C AgauCAA
194	CUUAUUU C AAUGgGA	399	CAAUGUU C AGAUCAA

399	CaAuGUU c	agAUCAA	658	CAGAUAU c	AcaagAu
399	caAUGUU c	aGAUCAA	658	CagauAU C	ACAAGAU
399	CAaUguU c	aGAUcAa	658	CAGauAU C	aCAAGAU
399	cAAuGuU C	aGAUcAA	658	CaGAUaU c	ACaAGau
399	CAaugUU c	agAUcAA	666	aCAAGAU A	AUGUCAC
404	UUCAGAU C	AAGGACA	666	ACAagaU a	AUGucAC
404	UucAGaU c	aAGGACa	671	AUaAuGU C	ACAGaAc
418	aUGgGCU c	GUAugAU	671	aUAAUgU c	ACAGAAc
418	AuGGGCU c	GUAugAu	671	AUAAUGU C	ACAGAAC
418	AUggGCU c	GUaUGaU	682	gAACUgU u	cAGUAUc
421	gGCUCgU a	UGAuugU	683	aAcUGuU c	aGuAUcU
421	ggCUCgU A	UgAuUGU	683	AAcUGuU c	agUaUcU
429	UgAuUGU u	UuAUaCA	691	aguaUcU C	CAaCAGC
429	UGAUuGU u	UUAUaCA	691	agUAUcU c	CAaCagc
431	AuUgUuU u	AUAcAAA	691	aGUAucU C	CAaCAGc
431	AUuGUuU U	AUAcAAa	701	aCaGCcU c	UcUCUuU
432	UuGUuUU A	UaCAaAA	701	acagCCU c	UCUCUuU
432	UuGUUUU a	UacaaAA	703	AGCcUcU C	UcUUUCA
432	uUGUUUU a	uAcaAAA	703	aGCcUcU c	UCUuUca
461	gAUcaAU u	AUCCucC	707	UcUCUcU U	UCAUUKC
462	AucaAUU a	uCcUCCA	707	UcUCUcU u	UcAUUCC
464	CAauUaU c	CUcCaAc	708	cUCUcU U	CAUUCcC
467	uUAUCcU C	CAaCagA	709	UCUcUuU C	AUUCcCg
467	UUauCcU C	CAaCAGA	709	UCUCUuU c	auuCCcG
467	UUaUccU c	cAACAGA	709	UCUcUuU c	AUUCcCg
467	UuAuCCU C	CaaCAGA	712	CUUucaU U	CcCgGaU
490	GAACUGU C	AGUGaUc	712	cuuUCAU U	cCCgGAU
497	CAGUGaU c	GCcAACTU	712	CuUucAU u	CcCGGaU
505	GCcAACTU U	CAGUGAA	712	cUUUCAU U	CCCgGAU
506	CcAACTU C	AGUGAAC	712	CUUUCAU u	ccCggaU
506	CCAaCTU C	aGUGaaC	713	uuUCAU U	CCgGAUg
521	CUGAAAU A	aaACugg	713	UUUCAU U	CCgGAUG
531	ACUGgcU c	AgAaUgU	732	GuGgcAU a	UGACcGU
539	agaaUGU A	ACAGGaa	732	GuGgcAU A	UGACCgU
550	GgAaAuU c	uGGCAuA	740	UGACCgU u	gUgUGUg
550	ggAAaUU C	UggCAUA	749	UgUGUgU U	CUGGAAA
557	cuggCAU A	AAUUUGA	749	uGuGUGU U	cUggAAA
561	CAUAAAU U	UGACCUG	750	gUGUGUU C	UGGAAAC
562	AUAAAUU U	GACCUGC	750	GuGUGUU c	UggAAAc
576	CaCgUCTU A	agCAaGG	773	ugAAGaU U	UcUcCa
585	gCAaGGU c	ACCCgaA	778	aUUUcCU c	caAAACCU
597	gaAACCU A	AGAAGAU	788	AAcUcCU C	AAuuuCA
607	AaGaUgU a	uUuUCUg	798	UUUCaCU c	aAGAGuU
611	UGUaUUU u	cUgAuAa	805	CAagAGU U	UccAUcu
625	AcuAAAU C	AACUAau	805	CAagAGU U	uccAUcU
630	UUCAACTU A	auGAGUA	806	AAgAGU U	ccAUcUc
630	UUcAAcU A	AuGAGUA	811	UUUCCA U	ucCUcaa
637	AauGAGU A	UGgUGaU	811	uUUCaU c	UcCUcaA
656	uGCAGaU a	UcAcAAG	813	uCCAUCU c	CUcaAac

836 aGgAGAU U acAGCUU  
836 aggaGAU U ACAGCUu  
837 GgAGAUU a cAGCUUc  
848 CUUCAGU u AcugUGg  
860 UGGCCcU C CUcCUug  
860 UggCCcU c CUCcuUg  
878 ugCUGCU C AUCauUg  
951 GCGGgaU a GuAACgC  
974 AgaCuAU c aACCUGA  
989 aGgaAcU U GaACCCc  
1006 auUgCUU c aGCAAAa  
1055 AAAgAGU u aaAAaUU  
1056 AaGAgUU a aaAAuUG  
1062 uAAAAAU u gcUuUgC  
1092 CAgaGUU u CuCAGAA  
1095 aGUUUcU c AgAaUUC  
1101 UCAGAAU u caaAaAU  
1101 ucAGAAU U CAAaaAU  
1101 UcAgAaU U CaAAaAu  
1111 aAaAUGU U cUcAgcU  
1112 AaAUGUU c UcAgcUg  
1128 UUGGAaU u cuACAGU  
1128 UUGGAaU u CuaCaGU  
1131 GAAuUCU a cAGuUgA  
1131 GAauUCU a CAguuGA  
1141 GuUGAAU a aUuAAag  
1144 gaaUAAU U AAAGAac  
1145 AAuAaUU a aAgaACA

Tabl BIX: Mouse B7-2 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequences
47	GUUGUUC CUGAUGAGGCCGAAAGGCCGAA AGUCCGU
47	GUUGUUC CUGAUGAGGCCGAAAGGCCGAA AGUCCGU
66	ACACGUC CUGAUGAGGCCGAAAGGCCGAA ACAGGAG
66	ACACGUC CUGAUGAGGCCGAAAGGCCGAA ACAGGAG
74	GUUCUGG CUGAUGAGGCCGAAAGGCCGAA ACACGUC
83	CUUCCGU CUGAUGAGGCCGAAAGGCCGAA AGUUCUG
134	CAAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGAUUG
134	CAAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGAUUG
134	CAAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGAUUG
134	CAAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGAUUG
134	CAAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGAUUG
134	CAAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGAUUG
135	ACAAAGA CUGAUGAGGCCGAAAGGCCGAA AAGGAUU
135	ACAAAGA CUGAUGAGGCCGAAAGGCCGAA AAGGAUU
135	ACAAAGA CUGAUGAGGCCGAAAGGCCGAA AAGGAUU
135	ACAAAGA CUGAUGAGGCCGAAAGGCCGAA AAGGAUU
137	UCACAAA CUGAUGAGGCCGAAAGGCCGAA AUAAGGA
137	UCACAAA CUGAUGAGGCCGAAAGGCCGAA AUAAGGA
137	UCACAAA CUGAUGAGGCCGAAAGGCCGAA AUAAGGA
139	UGUCACA CUGAUGAGGCCGAAAGGCCGAA AGAUAAG
140	CUGUCAC CUGAUGAGGCCGAAAGGCCGAA AAGAUA
140	CUGUCAC CUGAUGAGGCCGAAAGGCCGAA AAGAUA
149	UCAGCAA CUGAUGAGGCCGAAAGGCCGAA ACUGUCA
151	GAUCAGC CUGAUGAGGCCGAAAGGCCGAA AGACUGU
151	GAUCAGC CUGAUGAGGCCGAAAGGCCGAA AGACUGU
158	CAUCUGA CUGAUGAGGCCGAAAGGCCGAA AUCAGCA
158	CAUCUGA CUGAUGAGGCCGAAAGGCCGAA AUCAGCA
158	CAUCUGA CUGAUGAGGCCGAAAGGCCGAA AUCAGCA
158	CAUCUGA CUGAUGAGGCCGAAAGGCCGAA AUCAGCA
160	AGCAUCU CUGAUGAGGCCGAAAGGCCGAA AGAUCAG
160	AGCAUCU CUGAUGAGGCCGAAAGGCCGAA AGAUCAG
170	CCACGGA CUGAUGAGGCCGAAAGGCCGAA ACAGCAU
171	UCCACGG CUGAUGAGGCCGAAAGGCCGAA AACAGCA
172	CUCCACG CUGAUGAGGCCGAAAGGCCGAA AAACAGC
189	UUGAAAU CUGAUGAGGCCGAAAGGCCGAA AGCUUGC
189	UUGAAAU CUGAUGAGGCCGAAAGGCCGAA AGCUUGC
189	UUGAAAU CUGAUGAGGCCGAAAGGCCGAA AGCUUGC
190	AUUGAAA CUGAUGAGGCCGAAAGGCCGAA AAGCUUG
190	AUUGAAA CUGAUGAGGCCGAAAGGCCGAA AAGCUUG
192	CCAUUGA CUGAUGAGGCCGAAAGGCCGAA AUAAGCU
192	CCAUUGA CUGAUGAGGCCGAAAGGCCGAA AUAAGCU
193	CCCAUUG CUGAUGAGGCCGAAAGGCCGAA AAUAAGC
193	CCCAUUG CUGAUGAGGCCGAAAGGCCGAA AAUAAGC
194	UCCCAUU CUGAUGAGGCCGAAAGGCCGAA AAUAAG



194 UCCCAU CUGAUGAGGCCGAAAGGCCGAA AAAUAAG  
208 CGGCAGA CUGAUGAGGCCGAAAGGCCGAA AUGCAGU  
210 CACGGCA CUGAUGAGGCCGAAAGGCCGAA AUUAUGCA  
223 CUUUGUA CUGAUGAGGCCGAAAGGCCGAA AUGGGCA  
223 CUUUGUA CUGAUGAGGCCGAAAGGCCGAA AUGGGCA  
224 CCUUUGU CUGAUGAGGCCGAAAGGCCGAA AAUGGGC  
225 GCCUUUG CUGAUGAGGCCGAAAGGCCGAA AAAUGGG  
225 GCCUUUG CUGAUGAGGCCGAAAGGCCGAA AAAUGGG  
242 UCAGGCU CUGAUGAGGCCGAAAGGCCGAA AUGUUUU  
260 AAAAUAC CUGAUGAGGCCGAAAGGCCGAA ACCAGCU  
260 AAAAUAC CUGAUGAGGCCGAAAGGCCGAA ACCAGCU  
263 GCCAAA CUGAUGAGGCCGAAAGGCCGAA ACTUACCA  
263 GCCAAA CUGAUGAGGCCGAAAGGCCGAA ACTUACCA  
265 CUGCCAA CUGAUGAGGCCGAAAGGCCGAA AUACUAC  
265 CUGCCAA CUGAUGAGGCCGAAAGGCCGAA AUACUAC  
266 CCUGCCA CUGAUGAGGCCGAAAGGCCGAA AAUACTUA  
266 CCUGCCA CUGAUGAGGCCGAAAGGCCGAA AAUACTUA  
266 CCUGCCA CUGAUGAGGCCGAAAGGCCGAA AAUACTUA  
267 UCCUGCC CUGAUGAGGCCGAAAGGCCGAA AAUAUCTU  
267 UCCUGCC CUGAUGAGGCCGAAAGGCCGAA AAUAUCTU  
286 CAGAACC CUGAUGAGGCCGAAAGGCCGAA ACUUUUG  
286 CAGAACC CUGAUGAGGCCGAAAGGCCGAA ACUUUUG  
290 CGUACAG CUGAUGAGGCCGAAAGGCCGAA ACCAUCTU  
291 UCGUACA CUGAUGAGGCCGAAAGGCCGAA AACCAAC  
295 GUGCUUG CUGAUGAGGCCGAAAGGCCGAA ACAGAAC  
304 GCCCAA CUGAUGAGGCCGAAAGGCCGAA AGUGCTUC  
307 UGUGCCC CUGAUGAGGCCGAAAGGCCGAA AAUAGUG  
323 CACUAUC CUGAUGAGGCCGAAAGGCCGAA AGUUUCU  
343 GCCCAGG CUGAUGAGGCCGAAAGGCCGAA ACTUGGC  
343 GCCCAGG CUGAUGAGGCCGAAAGGCCGAA ACTUGGC  
361 CCUGUCA CUGAUGAGGCCGAAAGGCCGAA AGCUCGU  
381 AGUCGUA CUGAUGAGGCCGAAAGGCCGAA AGUCCAG  
383 GAAGUCG CUGAUGAGGCCGAAAGGCCGAA AGAGUCC  
383 GAAGUCG CUGAUGAGGCCGAAAGGCCGAA AGAGUCC  
389 CAUUGUG CUGAUGAGGCCGAAAGGCCGAA AGUUGUA  
389 CAUUGUG CUGAUGAGGCCGAAAGGCCGAA AGUUGUA  
390 ACAUUGU CUGAUGAGGCCGAAAGGCCGAA AAGUCGU  
390 ACAUUGU CUGAUGAGGCCGAAAGGCCGAA AAGUCGU  
398 UGAUCUG CUGAUGAGGCCGAAAGGCCGAA ACAUUGU  
398 UGAUCUG CUGAUGAGGCCGAAAGGCCGAA ACAUUGU  
398 UGAUCUG CUGAUGAGGCCGAAAGGCCGAA ACAUUGU  
399 UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AACADUG  
399 UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AACADUG  
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399 UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AACADUG  
399 UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AACADUG  
404 UGUCCUU CUGAUGAGGCCGAAAGGCCGAA AUCTUGAA

404 UGUCCUU CUGAUGAGGCCGAAAGGCCGAA AUCUGAA  
418 AUCAUAC CUGAUGAGGCCGAAAGGCCGAA AGCCCAU  
418 AUCAUAC CUGAUGAGGCCGAAAGGCCGAA AGCCCAU  
418 AUCAUAC CUGAUGAGGCCGAAAGGCCGAA AGCCCAU  
421 ACAAUCA CUGAUGAGGCCGAAAGGCCGAA ACGAGCC  
421 ACAAUCA CUGAUGAGGCCGAAAGGCCGAA ACGAGCC  
429 UGUUAUA CUGAUGAGGCCGAAAGGCCGAA ACAAUCA  
429 UGUUAUA CUGAUGAGGCCGAAAGGCCGAA ACAAUCA  
431 UUUGUUA CUGAUGAGGCCGAAAGGCCGAA AAACAAU  
431 UUUGUUA CUGAUGAGGCCGAAAGGCCGAA AAACAAU  
432 UUUUGUA CUGAUGAGGCCGAAAGGCCGAA AAAACAA  
432 UUUUGUA CUGAUGAGGCCGAAAGGCCGAA AAAACAA  
432 UUUUGUA CUGAUGAGGCCGAAAGGCCGAA AAAACAA  
461 GGAGGAU CUGAUGAGGCCGAAAGGCCGAA AUUGAUC  
462 UGGAGGA CUGAUGAGGCCGAAAGGCCGAA AAUUGAU  
464 GUUGGAG CUGAUGAGGCCGAAAGGCCGAA AUAUUG  
467 UCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGGAUAA  
467 UCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGGAUAA  
467 UCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGGAUAA  
467 UCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGGAUAA  
490 GAUCACU CUGAUGAGGCCGAAAGGCCGAA ACAGUUC  
497 AGUUGGC CUGAUGAGGCCGAAAGGCCGAA AUCACUG  
505 UUCACUG CUGAUGAGGCCGAAAGGCCGAA AGUUGGC  
506 GUUCACU CUGAUGAGGCCGAAAGGCCGAA AAGUUGG  
506 GUUCACU CUGAUGAGGCCGAAAGGCCGAA AAGUUGG  
521 CCAGUUU CUGAUGAGGCCGAAAGGCCGAA AUUUCAG  
531 ACAUUCU CUGAUGAGGCCGAAAGGCCGAA AGCCAGU  
539 UUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUCU  
550 UAUUGCA CUGAUGAGGCCGAAAGGCCGAA AAUUUCC  
550 UAUUGCA CUGAUGAGGCCGAAAGGCCGAA AAUUUCC  
557 UCAAAUU CUGAUGAGGCCGAAAGGCCGAA AUGCCAG  
561 CAGGUCA CUGAUGAGGCCGAAAGGCCGAA AUUUUAG  
562 GCAGGUC CUGAUGAGGCCGAAAGGCCGAA AAUUUUA  
576 CCTUGCU CUGAUGAGGCCGAAAGGCCGAA AGACGUG  
585 UUCGGGU CUGAUGAGGCCGAAAGGCCGAA ACCUUGC  
597 AUCUUCU CUGAUGAGGCCGAAAGGCCGAA AGGUTUC  
607 CAGAAAA CUGAUGAGGCCGAAAGGCCGAA ACAUUCU  
611 UUAUCAG CUGAUGAGGCCGAAAGGCCGAA AAAUACA  
625 AUUAGUU CUGAUGAGGCCGAAAGGCCGAA AAUAGU  
630 UACUCAU CUGAUGAGGCCGAAAGGCCGAA AGUUGAA  
630 UACUCAU CUGAUGAGGCCGAAAGGCCGAA AGUUGAA  
637 AUCACCA CUGAUGAGGCCGAAAGGCCGAA ACUCAU  
656 CUUGUGA CUGAUGAGGCCGAAAGGCCGAA AUCUGCA  
658 AUCUUGU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG  
658 AUCUUGU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG  
658 AUCUUGU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG  
658 AUCUUGU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG  
666 GUGACAU CUGAUGAGGCCGAAAGGCCGAA AUCUUGU  
666 GUGACAU CUGAUGAGGCCGAAAGGCCGAA AUCUUGU

671 GUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUAU  
671 GUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUAU  
671 GUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUAU  
682 GAUACUG CUGAUGAGGCCGAAAGGCCGAA ACAGUUC  
683 AGAUACU CUGAUGAGGCCGAAAGGCCGAA AACAGUU  
683 AGAUACU CUGAUGAGGCCGAAAGGCCGAA AACAGUU  
691 GCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGAUACU  
691 GCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGAUACU  
691 GCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGAUACU  
701 AAAGAGA CUGAUGAGGCCGAAAGGCCGAA AGGCUGU  
701 AAAGAGA CUGAUGAGGCCGAAAGGCCGAA AGGCUGU  
703 UGAAAGA CUGAUGAGGCCGAAAGGCCGAA AGAGGCU  
703 UGAAAGA CUGAUGAGGCCGAAAGGCCGAA AGAGGCU  
707 GGAAUGA CUGAUGAGGCCGAAAGGCCGAA AGAGAGA  
707 GGAAUGA CUGAUGAGGCCGAAAGGCCGAA AGAGAGA  
708 GGGAAUG CUGAUGAGGCCGAAAGGCCGAA AAGAGAG  
709 CGGGAAU CUGAUGAGGCCGAAAGGCCGAA AAAGAGA  
709 CGGGAAU CUGAUGAGGCCGAAAGGCCGAA AAAGAGA  
709 CGGGAAU CUGAUGAGGCCGAAAGGCCGAA AAAGAGA  
712 AUCCGGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAG  
712 AUCCGGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAG  
712 AUCCGGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAG  
712 AUCCGGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAG  
712 AUCCGGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAG  
713 CAUCCGG CUGAUGAGGCCGAAAGGCCGAA AAUGAAA  
713 CAUCCGG CUGAUGAGGCCGAAAGGCCGAA AAUGAAA  
732 ACGGUCA CUGAUGAGGCCGAAAGGCCGAA AUGCCAC  
732 ACGGUCA CUGAUGAGGCCGAAAGGCCGAA AUGCCAC  
740 CACACAC CUGAUGAGGCCGAAAGGCCGAA ACGGUCA  
749 UUUCCAG CUGAUGAGGCCGAAAGGCCGAA ACACACA  
749 UUUCCAG CUGAUGAGGCCGAAAGGCCGAA ACACACA  
750 GUUCCA CUGAUGAGGCCGAAAGGCCGAA AACACAC  
750 GUUCCA CUGAUGAGGCCGAAAGGCCGAA AACACAC  
773 UGGAGGA CUGAUGAGGCCGAAAGGCCGAA AUCUUCA  
778 AGGUUUG CUGAUGAGGCCGAAAGGCCGAA AGGAAAU  
788 UGAAAUU CUGAUGAGGCCGAAAGGCCGAA AGAGGUU  
798 AACUCUU CUGAUGAGGCCGAAAGGCCGAA AGUGAAA  
805 AGAUGGA CUGAUGAGGCCGAAAGGCCGAA ACUCUUG  
805 AGAUGGA CUGAUGAGGCCGAAAGGCCGAA ACUCUUG  
806 GAGAUGG CUGAUGAGGCCGAAAGGCCGAA AACUCUU  
811 UUGAGGA CUGAUGAGGCCGAAAGGCCGAA AUGGAAA  
811 UUGAGGA CUGAUGAGGCCGAAAGGCCGAA AUGGAAA  
813 GUUUGAG CUGAUGAGGCCGAAAGGCCGAA AGAUGGA  
836 AAGCUGU CUGAUGAGGCCGAAAGGCCGAA AUCUCCU  
836 AAGCUGU CUGAUGAGGCCGAAAGGCCGAA AUCUCCU  
837 GAAGCUG CUGAUGAGGCCGAAAGGCCGAA AAUCUCC  
848 CCACAGU CUGAUGAGGCCGAAAGGCCGAA ACUGAAG  
860 CAAGGAG CUGAUGAGGCCGAAAGGCCGAA AGGGCCA  
860 CAAGGAG CUGAUGAGGCCGAAAGGCCGAA AGGGCCA

878	CAAUGAU	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCA
951	GCGUUC	CUGAUGAGGCCGAAAGGCCGAA	AUCCCGC
974	UCAGGUU	CUGAUGAGGCCGAAAGGCCGAA	AUAGUCU
989	GGGUUC	CUGAUGAGGCCGAAAGGCCGAA	AGUUCU
1006	UUUUGCU	CUGAUGAGGCCGAAAGGCCGAA	AAGCAAU
1055	AAUUUUU	CUGAUGAGGCCGAAAGGCCGAA	ACUCUUU
1056	CAUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AACUCUU
1062	GCAAAGC	CUGAUGAGGCCGAAAGGCCGAA	AUUUUUA
1092	UUCUGAG	CUGAUGAGGCCGAAAGGCCGAA	AACUCUG
1095	GAAUUCU	CUGAUGAGGCCGAAAGGCCGAA	AGAAACU
1101	AUUUUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCUGA
1101	AUUUUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCUGA
1101	AUUUUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCUGA
1111	AGCUGAG	CUGAUGAGGCCGAAAGGCCGAA	ACAUUUU
1112	CAGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AACAUUU
1128	ACUGUAG	CUGAUGAGGCCGAAAGGCCGAA	AUUCCAA
1128	ACUGUAG	CUGAUGAGGCCGAAAGGCCGAA	AUUCCAA
1131	UCAACUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAUUC
1131	UCAACUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAUUC
1141	CUUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AUUC AAC
1144	GUUCUUU	CUGAUGAGGCCGAAAGGCCGAA	AUUAUUC
1145	UGUUCUU	CUGAUGAGGCCGAAAGGCCGAA	AAUUAUU

Table BX: Human CD40 Hammerhead Ribozyme Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
9	CCUUGCU C GGGCGCC	440	UUGGGGU C AAGCAGA
24	CAGUGGU C CUGCGGC	449	AGCAGAU U GCUACAG
37	GCCUGGU C UCACCU C	453	GAUUGCU A CAGGGGU
39	CUGGUU C ACCUUGC	461	CAGGGGU U UCUGAUA
44	CUCACCU C GCCAUGG	462	AGGGGUU U CUGAUAC
53	CCAUGGU U CGUCUGC	463	GGGGUUU C UGAUACC
54	CAUGGUU C GUCUGCC	468	UUCUGAU A CCAUCUG
57	GGUUCGU C UGCTUCU	473	AUACCAU C UGCGAGC
63	UCUGCCU C UGCAGUG	491	GCCCAGU C GGUUCU
74	AGUGCGU C CUCUGGG	496	GUCGGCU U CUUCUCC
77	GCGUCCU C UGGGGCU	497	UCGGCUU C UUCUCCA
88	GGCUGCU U GCUAGCC	499	GGCUUCU U CUCCAAU
101	CCGCTUGU C CAUCCAG	500	GCUUCUU C UCCAAUG
105	UGUCCAU C CAGAAC	502	UUCUUCU C CAAUGUG
139	AAACAGU A CUAUAUA	511	AAUGUGU C AUCUGCU
143	AGUACCU A AUAAACA	514	GUGUCAU C UGCUUUC
146	ACCUAAU A AACAGUC	519	AUCUGCU U UCGAAAA
153	AAACAGU C AGUGCUG	520	UCUGCUU U CGAAAAA
162	GUGCUGU U CUUUGUG	521	CUGCUUU C GAAAAAU
163	UGCUGUU C UUUGUGC	531	AAAAUGU C ACCCUUG
165	CUGUUCU U UGUGCCA	537	UCACCCU U GGACAAG
166	UGUUCUU U GUGCCAG	566	ACCUGGU U GUGCAAC
208	ACAGAGU U CACUGAA	599	CUGAUGU U GUCUGUG
209	CAGAGUU C ACUGAAA	602	AUGUUGU C UGUGGUC
227	AAUGCCU U CCUUGCG	609	CUGUGGU C CCCAGGA
228	AUGCCUU C CUUGCGG	618	CCAGGAU C GGCUGAG
231	CCUUCUU U GCGGUGA	641	UGGUGAU C CCCAUCA
247	AGCGAAU U CCUAGAC	647	UCCCCAU C AUCUUCG
248	GCGAAUU C CUAGACA	650	CCAUCAU C UUCGGGA
251	AAUUCUU A GACACCU	652	AUCAUCU U CGGGAUC
292	CACAAAU A CUGCGAC	653	UCAUCUU C GGGAUCC
308	CCAACCU A GGGCUUC	659	UCGGGAU C CUGUUUG
314	UAGGGCU U CGGGUCC	664	AUCCUGU U UGCCAUC
315	AGGGCUU C GGGUCCA	665	UCCUGUU U GCCAUCC
320	UUCGGGU C CAGCAGA	671	UUGCCAU C CUCUUGG
337	GGCACCU C AGAAACA	674	CCAUCUU C UUGGUGC
353	ACACCAU C UGCACCU	676	AUCCUUU U GGUGCUG
381	GCAUGU A CGAGUGA	686	UGCUGGU C UUAUCA
407	GUCUGU C CUGCACC	688	CUGGUU U UAUCAAA
418	CACCGCU C AUGCUGG	689	UGGUUUU U AUCAAAA
424	UCAUGCU C GCCCGGC	690	GGUUCUU A UCAAAAA
433	CCCGGCU U UGGGGUC	692	UCUUUUAU C AAAAAGG
434	CCGGCUU U GGGGUCA	720	AACCAAU A AGGCCCC

755	AGGAGAU C AAUUUUC
759	GAUCAAU U UUCCCGA
760	AUCAAUU U UCCCGAC
761	UCAAUUU U CCCGACG
762	CAAUUUU C CCGACGA
771	CGACGAU C UUCCUGG
773	ACGAUCU U CCUGGCU
774	CGAUCUU C CUGGCUC
781	CCUGGCU C CAACACU
795	UGCUGCU C CAGUGCA
810	GGAGACTU U UACAUGG
811	GAGACTU U ACAUGGA
812	AGACTUU A CAUGGAU
830	AACCGGU C ACCCAGG
855	AGAGAGU C GCAUCUC
860	GUCGCAU C UCAGUGC
862	CGCAUCU C AGUGCAG
927	AGGCAGU U GGCCAGA
981	GGGAGCU A UGCCCAG
990	GCCCAGU C AGUGCCA

Table BXL: Human CD40 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequences
9	GGCGCCC CUGAUGAGGCCGAAAGGCCGAA AGCGAGG
24	GCGGCAG CUGAUGAGGCCGAAAGGCCGAA ACCACUG
37	GAGGUGA CUGAUGAGGCCGAAAGGCCGAA ACCAGGC
39	GCGAGGU CUGAUGAGGCCGAAAGGCCGAA AGACCAG
44	CCAUGGC CUGAUGAGGCCGAAAGGCCGAA AGGUGAG
53	GCAGACG CUGAUGAGGCCGAAAGGCCGAA ACCAUGG
54	GGCAGAC CUGAUGAGGCCGAAAGGCCGAA AACCAUG
57	AGAGGCA CUGAUGAGGCCGAAAGGCCGAA ACGAACC
63	CAUGCA CUGAUGAGGCCGAAAGGCCGAA AGGCAGA
74	CCCAGAG CUGAUGAGGCCGAAAGGCCGAA ACGCACU
77	AGCCCCA CUGAUGAGGCCGAAAGGCCGAA AGGACGC
88	GGUCAGC CUGAUGAGGCCGAAAGGCCGAA AGCAGCC
101	CUGGAUG CUGAUGAGGCCGAAAGGCCGAA ACAGCGG
105	GGUUCUG CUGAUGAGGCCGAAAGGCCGAA AUGGACA
139	UAUUAGG CUGAUGAGGCCGAAAGGCCGAA ACUGUUU
143	UGUUUAU CUGAUGAGGCCGAAAGGCCGAA AGGUACU
146	GACUGUU CUGAUGAGGCCGAAAGGCCGAA AUUAGGU
153	CAGCACU CUGAUGAGGCCGAAAGGCCGAA ACUGUUU
162	CACAAAG CUGAUGAGGCCGAAAGGCCGAA ACAGCAC
163	GCACAA CUGAUGAGGCCGAAAGGCCGAA AACAGCA
165	UGGCACA CUGAUGAGGCCGAAAGGCCGAA AGAACAG
166	CUGGCAC CUGAUGAGGCCGAAAGGCCGAA AAGAACA
208	UUCAGUG CUGAUGAGGCCGAAAGGCCGAA ACTUCUG
209	UUUCAGU CUGAUGAGGCCGAAAGGCCGAA AACTUCG
227	CGCAAGG CUGAUGAGGCCGAAAGGCCGAA AGGCAUU
228	CCGCAAG CUGAUGAGGCCGAAAGGCCGAA AAGGCAU
231	UCACCGC CUGAUGAGGCCGAAAGGCCGAA AGGAAGG
247	GUCUAGG CUGAUGAGGCCGAAAGGCCGAA AUUCGCU
248	UGUCUAG CUGAUGAGGCCGAAAGGCCGAA AAUUCGC
251	AGGUGUC CUGAUGAGGCCGAAAGGCCGAA AGGAAUJ
292	GUCGCAG CUGAUGAGGCCGAAAGGCCGAA AUUUGUG
308	GAAGCCC CUGAUGAGGCCGAAAGGCCGAA AGGUUGG
314	GGACCCG CUGAUGAGGCCGAAAGGCCGAA AGCCCUA
315	UGGACCC CUGAUGAGGCCGAAAGGCCGAA AAGCCCU
320	UCUGCUG CUGAUGAGGCCGAAAGGCCGAA ACCCGAA
337	UGUUUTU CUGAUGAGGCCGAAAGGCCGAA AGGUGCC
353	AGGUGCA CUGAUGAGGCCGAAAGGCCGAA AUGGUGU
381	UACUCG CUGAUGAGGCCGAAAGGCCGAA ACAGUGC
407	GGUGCAG CUGAUGAGGCCGAAAGGCCGAA ACACAGC
418	CGAGCAU CUGAUGAGGCCGAAAGGCCGAA AGCGGUG
424	GCCGGGC CUGAUGAGGCCGAAAGGCCGAA AGCAUGA
433	GACCCCA CUGAUGAGGCCGAAAGGCCGAA AGCCGGG
434	UGACCCC CUGAUGAGGCCGAAAGGCCGAA AAGCCGG

440	UCUGCUU	CUGAUGAGGCCGAAAGGCCGAA	ACCCCAA
449	CUGUAGC	CUGAUGAGGCCGAAAGGCCGAA	AUCUGCU
453	ACCCCTUG	CUGAUGAGGCCGAAAGGCCGAA	AGCAAUC
461	UAUCAGA	CUGAUGAGGCCGAAAGGCCGAA	ACCCCTUG
462	GUUUCAG	CUGAUGAGGCCGAAAGGCCGAA	AACCCCU
463	GGUAUCA	CUGAUGAGGCCGAAAGGCCGAA	AAACCCC
468	CAGAUUG	CUGAUGAGGCCGAAAGGCCGAA	AUCAGAA
473	GCUUGCA	CUGAUGAGGCCGAAAGGCCGAA	AUGGUUU
491	AGAAGCC	CUGAUGAGGCCGAAAGGCCGAA	ACUGGGC
496	GGAGAAG	CUGAUGAGGCCGAAAGGCCGAA	AGCCGAC
497	UGGAGAA	CUGAUGAGGCCGAAAGGCCGAA	AAGCCGA
499	AUUGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGAAGCC
500	CAUUGGA	CUGAUGAGGCCGAAAGGCCGAA	AAGAAGC
502	CACAUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAGAA
511	AGCAGAU	CUGAUGAGGCCGAAAGGCCGAA	ACACAUU
514	GAAAGCA	CUGAUGAGGCCGAAAGGCCGAA	AUGACAC
519	UUUUCGA	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAU
520	UUUUUCG	CUGAUGAGGCCGAAAGGCCGAA	AAGCAGA
521	AUUUUUC	CUGAUGAGGCCGAAAGGCCGAA	AAAGCAG
531	CAAGGGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUUU
537	CUUGUCC	CUGAUGAGGCCGAAAGGCCGAA	AGGGUGA
566	GUUGCAC	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGU
599	CACAGAC	CUGAUGAGGCCGAAAGGCCGAA	ACAUCAG
602	GACCACA	CUGAUGAGGCCGAAAGGCCGAA	ACAACAU
609	UCCUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACCACAG
618	CUCAGCC	CUGAUGAGGCCGAAAGGCCGAA	AUCCUGG
641	UGAUGGG	CUGAUGAGGCCGAAAGGCCGAA	AUCACCA
647	CGAAGAU	CUGAUGAGGCCGAAAGGCCGAA	AUGGGGA
650	UCCCGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGAUGG
652	GAUCCCG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGAU
653	GGAUCCC	CUGAUGAGGCCGAAAGGCCGAA	AAGAUGA
659	CAAACAG	CUGAUGAGGCCGAAAGGCCGAA	AUCCCGA
664	GAUGGCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGGAU
665	GGAUGGC	CUGAUGAGGCCGAAAGGCCGAA	AACAGGA
671	CCAAGAG	CUGAUGAGGCCGAAAGGCCGAA	AUGGCAA
674	GCACCAA	CUGAUGAGGCCGAAAGGCCGAA	AGGAUGG
676	CAGCACC	CUGAUGAGGCCGAAAGGCCGAA	AGAGGAU
686	UGAUAAA	CUGAUGAGGCCGAAAGGCCGAA	ACCAGCA
688	UUUGAUA	CUGAUGAGGCCGAAAGGCCGAA	AGACCAG
689	UUUUGAU	CUGAUGAGGCCGAAAGGCCGAA	AAGACCA
690	UUUUUGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGACC
692	CTUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AUAAAGA
720	GGGGCTU	CUGAUGAGGCCGAAAGGCCGAA	AUUGGUU
755	GAAAAUU	CUGAUGAGGCCGAAAGGCCGAA	AUCUCCU
759	UCGGGAA	CUGAUGAGGCCGAAAGGCCGAA	AUUGAUC
760	GUCGGGA	CUGAUGAGGCCGAAAGGCCGAA	AAUUGAU
761	CGUCGGG	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGA
762	UCGUCGG	CUGAUGAGGCCGAAAGGCCGAA	AAAAUUG
771	CCAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AUCGUCC



773	AGCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGAUCGU
774	GAGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AAGAUCG
781	AGUGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCAGG
795	UGCACUG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCA
810	CCAUGUA	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCC
811	UCCAUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGUCUC
812	AUCCAUG	CUGAUGAGGCCGAAAGGCCGAA	AAAGUCU
830	CCUGGGU	CUGAUGAGGCCGAAAGGCCGAA	ACCGGUU
855	GAGAUCC	CUGAUGAGGCCGAAAGGCCGAA	ACUCUCU
860	GCACUGA	CUGAUGAGGCCGAAAGGCCGAA	AUGCGAC
862	CUGCACU	CUGAUGAGGCCGAAAGGCCGAA	AGAUGCG
927	UCUGGCC	CUGAUGAGGCCGAAAGGCCGAA	ACUGCCU
981	CUGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCC
990	UGGCACU	CUGAUGAGGCCGAAAGGCCGAA	ACUGGGC

Table BXII: Mouse CD40 Hammerhead Ribozyme Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
18	GGUgucU u UGCCUCg	479	cAUCACu U UUCgaaA
18	GGuguCU u UGCCucG	480	AUCacuU U UCGAAAA
24	UuUGCCU C gGCuGUG	481	UCacuUU U CGAAAAg
38	GCGcgCU a UGGGGCU	481	UCACuuU U cGaaAAg
62	CagcGGU c CaUCUag	492	AAAgUGU u AuCCcUG
62	CaGcGGU C CAUCuAG	560	CUaAUGU c aUCUGUG
66	gGUCCA U C uAGggCa	563	AUGUcaU C UGUGGuu
80	AGUGuGU u acgUGca	572	gUGGUuU a AagUCcC
80	AgUGUGU u AcgUGCa	572	GuGGUUU a aagUcCC
81	gUGugUU a CgUGCaG	577	UuAAagU c CCgGAuG
100	AAACAGU A CCUccac	620	UGGgcAU C CuCAUCA
126	CUGUgaU U UGUGCCA	626	UCCuCAU C AcCaUuu
127	UGUGaUU U GUGCCAG	632	uCAcCAU u UUCGGGg
170	CAGcUcU u gaGAaGA	632	UcaCCA U uUCgGGG
208	gGCGAAU U CUCAGcC	634	AcCAUuU U CGGGgUg
209	GCGAAUU C ucAGcCc	635	CCaUuuU c GgGGUGu
233	gGGAGAU u cgcUgUC	635	cCAUuUU C GGGgUgu
267	ACCCAAU c AAggGcu	635	CCAuuuU C ggGGUGu
267	AcCCAAU c AaGggCu	647	UGUuucU C UaUAUCA
275	aAGGGCU U CGGGUua	649	uUucUCU a UAUCAA
275	AaGGGcU U CgGgUua	651	ucUCUaU A UCAAAAA
276	AaGGGCUU C GGGUuaA	653	UCUaUAU C AAAAAGG
281	UUCGGGU u aAGaAGg	735	gGAaGAU u aUCCcGG
281	UUCGGGU u AAGaAGg	759	cGCUVCU C CAGUGCA
314	ACACugU C UGUACCU	794	AgCCuGU C ACaCAGG
354	caAgGaU u GCgaGGC	794	AGcCUGU c acaCAGg
386	cCugUaU c CCUGGCU	819	AGAGAGU C GCAUCUC
394	CCUgGCU u uGGaGuu	824	GUcGCAU C UCAGUGC
394	CCUGGCU U UGGaGUu	826	CGCAUCU C AGUGCAG
395	CuGGCTU U GGaGUua	876	cCTUGGU C UgAaCcC
429	caCUGAU A CCgUCUG	913	GGCUGCU U GCUGACC
434	AUAACG U C UGUcAuC	997	CUCAaCU u GCuuUuu
434	AUAcCGU c UGUCAUC	1003	uUGCTUU u uAaggAU
441	CugUCAU C CcuGCcC	1003	uugCTUU u uAaGGAU
452	GCCCAGU C GGCUCU	1023	gaAAgCU c GGGCaUC
452	GCCCAGU C gGcuuCu	1048	CAGuGAU a UCUacca
457	GUcGGCU U CUUCUCC	1052	gAUauCU a CCaaGuG
458	UCGGCTU C UUCUCCA	1081	CCAGagU u GuCUgc
460	GGCTUCU U CUCCAAU	1084	gAGUuGU C uUGCuGC
461	GCTUCUU C UCCAAUc	1086	gUugUcU U GcUGCgG
463	UUCUUCU C CAUcag	1097	gCgGcGU U CACTUGuA
472	AAuCAGU C AucaCUu	1098	CgGcGUU C ACUGuAA
472	AAUcagU c auCACuU	1118	cgUgGCU A CAGGaGU

1118	CgUGGCU a CAggAgU
1141	CgCaGCU u gUGCUCG
1164	aCCUGgU U GCCAUCa
1202	UGuaaUU a UUUAUaC
1220	gGcAuCU c AgAAACu
1220	GGCAuCU C AGAAACu
1228	aGAaACU c UAgaGG
1253	AaCaGGU a GUGgAAu
1331	AGgAGcU U GCUgCcc
1362	uUuUGaU C CCugGGA
1373	gGGaCUU c AUgguAA
1373	GgGACUU c AugguaA
1413	uUGUCAU u UGaccUC
1443	GUaaUGU a CcccGUG
1470	CACaUAU c CUaaaAu
1492	GugGUGU a uUGuAga
1497	GuAuUGU A gaAaUuA
1508	auUauUU a aUCCGCC
1508	AUuAuUU a auCCGcC
1523	cuGGGuU u CUaccUG

Tabl BXIII: Mouse CD40 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
18	CGAGGCA CUGAUGAGGCCGAAAGGCCGAA AGACACC
18	CGAGGCA CUGAUGAGGCCGAAAGGCCGAA AGACACC
24	CACAGCC CUGAUGAGGCCGAAAGGCCGAA AGGCAAA
38	AGCCCCA CUGAUGAGGCCGAAAGGCCGAA AGCGCGC
62	CUAGAUG CUGAUGAGGCCGAAAGGCCGAA ACCGCTUG
62	CUAGAUG CUGAUGAGGCCGAAAGGCCGAA ACCGCTUG
66	UGCCCTUA CUGAUGAGGCCGAAAGGCCGAA AUGGACC
80	UGCACGU CUGAUGAGGCCGAAAGGCCGAA ACACACU
80	UGCACGU CUGAUGAGGCCGAAAGGCCGAA ACACACU
81	CUGCACG CUGAUGAGGCCGAAAGGCCGAA AACACAC
100	GUGGAGG CUGAUGAGGCCGAAAGGCCGAA ACUGUUU
126	UGGCACA CUGAUGAGGCCGAAAGGCCGAA AUCACAG
127	CUGGCAC CUGAUGAGGCCGAAAGGCCGAA AAUCACA
170	UCUUCUC CUGAUGAGGCCGAAAGGCCGAA AGAGCUG
208	GGCUGAG CUGAUGAGGCCGAAAGGCCGAA AUUCGCC
209	GGGCUGA CUGAUGAGGCCGAAAGGCCGAA AAUUCGC
233	GACAGCG CUGAUGAGGCCGAAAGGCCGAA AUCUCCC
267	AGCCCTU CUGAUGAGGCCGAAAGGCCGAA AUUGGGU
267	AGCCCTU CUGAUGAGGCCGAAAGGCCGAA AUUGGGU
275	UAACCCG CUGAUGAGGCCGAAAGGCCGAA AGCCCTU
275	UAACCCG CUGAUGAGGCCGAAAGGCCGAA AGCCCTU
276	UUAACCC CUGAUGAGGCCGAAAGGCCGAA AAGCCCTU
281	CCTUUCU CUGAUGAGGCCGAAAGGCCGAA ACCCGAA
281	CCTUUCU CUGAUGAGGCCGAAAGGCCGAA ACCCGAA
314	AGGUACA CUGAUGAGGCCGAAAGGCCGAA ACAGUGU
354	GCTUCGC CUGAUGAGGCCGAAAGGCCGAA AUCCUUG
386	AGCCAGG CUGAUGAGGCCGAAAGGCCGAA AUACAGG
394	AACUCCA CUGAUGAGGCCGAAAGGCCGAA AGCCAGG
394	AACUCCA CUGAUGAGGCCGAAAGGCCGAA AGCCAGG
395	UAACUCC CUGAUGAGGCCGAAAGGCCGAA AAGCCAG
429	CAGACGG CUGAUGAGGCCGAAAGGCCGAA AUCAGUG
434	GAUGACA CUGAUGAGGCCGAAAGGCCGAA ACGGUUU
434	GAUGACA CUGAUGAGGCCGAAAGGCCGAA ACGGUUU
441	GGGCAGG CUGAUGAGGCCGAAAGGCCGAA AUGACAG
452	AGAAGCC CUGAUGAGGCCGAAAGGCCGAA ACUGGGC
452	AGAAGCC CUGAUGAGGCCGAAAGGCCGAA ACUGGGC
457	GGAGAAG CUGAUGAGGCCGAAAGGCCGAA AGCCGAC
458	UGGAGAA CUGAUGAGGCCGAAAGGCCGAA AAGCCGA
460	AUUGGAG CUGAUGAGGCCGAAAGGCCGAA AGAAGCC
461	GAUUGGA CUGAUGAGGCCGAAAGGCCGAA AAGAAGC
463	CUGAUUG CUGAUGAGGCCGAAAGGCCGAA AGAAGAA
472	AAGUGAU CUGAUGAGGCCGAAAGGCCGAA ACUGAUU
472	AAGUGAU CUGAUGAGGCCGAAAGGCCGAA ACUGAUU

479	UUUCGAA	CUGAUGAGGCCGAAAGGCCGAA	AGUGAUG
480	UUUUCGA	CUGAUGAGGCCGAAAGGCCGAA	AAGUGAU
481	CUUUUCG	CUGAUGAGGCCGAAAGGCCGAA	AAAGUGA
481	CUUUUCG	CUGAUGAGGCCGAAAGGCCGAA	AAAGUGA
492	CAGGGAU	CUGAUGAGGCCGAAAGGCCGAA	ACACUUU
560	CACAGAU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUAG
563	AACCACA	CUGAUGAGGCCGAAAGGCCGAA	AUGACAU
572	GGGACUU	CUGAUGAGGCCGAAAGGCCGAA	AAACCAC
572	GGGACUU	CUGAUGAGGCCGAAAGGCCGAA	AAACCAC
577	CAUCCGG	CUGAUGAGGCCGAAAGGCCGAA	ACUUUAA
620	UGAUGAG	CUGAUGAGGCCGAAAGGCCGAA	AUGCCCA
626	AAAUUGU	CUGAUGAGGCCGAAAGGCCGAA	AUGAGGA
632	CCCCGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGA
632	CCCCGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGA
634	CACCCCG	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGU
635	ACACCCC	CUGAUGAGGCCGAAAGGCCGAA	AAAAUUG
635	ACACCCC	CUGAUGAGGCCGAAAGGCCGAA	AAAAUUG
635	ACACCCC	CUGAUGAGGCCGAAAGGCCGAA	AAAAUUG
647	UGAUUAU	CUGAUGAGGCCGAAAGGCCGAA	AGAAACA
649	UUUGAUA	CUGAUGAGGCCGAAAGGCCGAA	AGAGAAA
651	UUUUUGA	CUGAUGAGGCCGAAAGGCCGAA	AUAGAGA
653	CCUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AUAUAGA
735	CCGGGAU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUCC
759	UGCACUG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCG
794	CCUGUGU	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCU
794	CCUGUGU	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCU
819	GAGAUUC	CUGAUGAGGCCGAAAGGCCGAA	ACUCUCU
824	GCACTGA	CUGAUGAGGCCGAAAGGCCGAA	AUGCGAC
826	CUGCACU	CUGAUGAGGCCGAAAGGCCGAA	AGAUGCG
876	GGGUUCA	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGG
913	GGUCAGC	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCC
997	AAAAAGC	CUGAUGAGGCCGAAAGGCCGAA	AGUUGAG
1003	AUCCUUA	CUGAUGAGGCCGAAAGGCCGAA	AAAGCAA
1003	AUCCUUA	CUGAUGAGGCCGAAAGGCCGAA	AAAGCAA
1023	GAUGCCC	CUGAUGAGGCCGAAAGGCCGAA	AGCUUUC
1048	UGGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AUCACUG
1052	CACUUGG	CUGAUGAGGCCGAAAGGCCGAA	AGAUUUC
1081	GCAAGAC	CUGAUGAGGCCGAAAGGCCGAA	ACUCUUG
1084	GCAGCAA	CUGAUGAGGCCGAAAGGCCGAA	ACAACUC
1086	CCGCAGC	CUGAUGAGGCCGAAAGGCCGAA	AGACAAC
1097	UACAGUG	CUGAUGAGGCCGAAAGGCCGAA	ACGCCGC
1098	UUACAGU	CUGAUGAGGCCGAAAGGCCGAA	AACGCCG
1118	ACUCCUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCACG
1118	ACUCCUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCACG
1141	CGAGCAC	CUGAUGAGGCCGAAAGGCCGAA	AGCUGCG
1164	UGAUGGC	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGU
1202	GUUUAUA	CUGAUGAGGCCGAAAGGCCGAA	AAUUAUA
1220	AGUUUCU	CUGAUGAGGCCGAAAGGCCGAA	AGAUGCC
1220	AGUUUCU	CUGAUGAGGCCGAAAGGCCGAA	AGAUGCC

1228	CCUGCUA	CUGAUGAGGCCGAAAGGCCGAA	AGUUTCU
1253	AUUCCAC	CUGAUGAGGCCGAAAGGCCGAA	ACCUGUU
1331	GGGCAGC	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCU
1362	UCCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUCAAAA
1373	UUACCAU	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCC
1373	UUACCAU	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCC
1413	GAGGUCA	CUGAUGAGGCCGAAAGGCCGAA	AUGACAA
1443	CACGGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAUUAC
1470	AUUUUAG	CUGAUGAGGCCGAAAGGCCGAA	AUAUGUG
1492	UCUACAA	CUGAUGAGGCCGAAAGGCCGAA	ACACCAC
1497	UAAUUTC	CUGAUGAGGCCGAAAGGCCGAA	ACAAUAC
1508	GGCGGAU	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAU
1508	GGCGGAU	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAU
1523	CAGGUAG	CUGAUGAGGCCGAAAGGCCGAA	AACCCAG

Table BXIV: Human B7 Hairpin Ribozyme and Target Sequence

nt. Position	Hairpin Ribozyme Sequence		Substrate
286	ACAGGCAG	AGAA GAUGAC	GUCAUCA
291	GCAAAACA	AGAA GGGCUG	GCC CUGCCUGU
295	AGGUGCAA	AGAA GGCAGG	GCC UGUUUUGC
437	GCACCAAG	AGAA GAAAGA	GUU UUGCACCUCU
469	AACACCTUG	AGAA GAAAGUG	GCU CUUGGGUGC
518	GACACACAG	AGAA GCGUUG	GCU CAGGUGGU
540	AGCUUCUC	AGAA GAAACA	GUC CUGUGGUC
596	ACAUCAUA	AGAA GCACCA	GUU GAAAGACU
644	CAAAGNUG	AGAA GGUUCU	GAC UAUGAUGU
702	GUGCCCTUC	AGAA GAUGGG	GAC CAUCUUUG
795	GUAGGGNA	AGAA GCUUUG	GAC GAGGGCAC
819	AUUUCANA	AGAA GAUUAU	GAC UUCUCCUAC
939	UCUUGGGA	AGAA GUUGUG	GAC UUGGNAAU
1012	ACAUAUGA	AGAA GUUGUU	GUU UCCCNAGA
1055	AGUUGAAG	AGAA GAUUCA	GCU UCAUGUGU
1103	AGGAUUGG	AGAA GGUUAU	GAC CUUCAACU
1159	GUAGGUCA	AGAA GCUUAU	GCU CCCAUCCU
1163	AGCAGUAG	AGAA GGCAGC	GCC UGACCCUAC
1171	UGGGGCCAA	AGAA GUAGGU	GAC CUACUCCU
1356	GUUGGGUAA	AGAA GCUUAA	GCU UUGCCCCA
1395	UCAGGCUUA	AGAA GAAAGA	GUU UUAUCCAC
			UCUUUCA GAU UAAGCTUGA

Table BXV: Mouse B7 Hairpin Ribozyme and Target Sequence

nt. Position	Hairpin Ribozyme Sequence				Substrate
74	AGAAUUG	AGAA	GAGUGU	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	ACACUCU GUU CCAUUDUCU
114	AUCCACCC	AGAA	GAUGCU	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AGCAUCU GCC GGGUGGAU
154	AAUUGAGA	AGAA	GAGAUG	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CAUCUCU GUU UCUCGGAU
265	CCUGCAUC	AGAA	GACAAU	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AUUGUCA GUU GAUGCAGG
328	GACGAUUC	AGAA	GCACAA	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UUUGUCU GCU GAUUCGUC
331	AAAGACGA	AGAA	GCAGCA	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UGCUGCU GAU UCGUCUUU
356	UCAUCUAC	AGAA	GAAGAC	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GUCUUCA GAU GUUGAUGA
373	CUGACUUG	AGAA	GUUGUU	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AACAACU GUC CAAGUCAG
403	AACGGCNA	AGAA	GCAUUA	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UAUUGCU GCC UUGCCGUU
481	CAAUGACA	AGAA	GCACCA	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UGGUGCU GUC UGUGAUUG
529	CAUAUAAA	AGAA	GGUUCU	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AGAACCG GAC UUUUAUUG
584	GUGCCCGG	AGAA	GAUAGG	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CCUUUCA GCU CGGGGCAC
600	NACGACAC	AGAA	GUUUGU	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	ACAUACA GCU GUUGUGUU
677	GUAGAGAA	AGAA	GCUIUG	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CMAAGCU GAC UUCUCTAC
741	GGAAACAA	AGAA	GGUAAU	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AUUAACCU GCU UUGCTUCC
1028	AUGAGCAC	AGAA	GUUAUU	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AAUAACA GUC GUUGUCAU
1077	UCUUCUGA	AGAA	GCUDCU	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AGAAGCU GUU UCAGAAGA
1116	GAAGGUAA	AGAA	GUUGUU	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AACAACA GCC UUAACCUUC
1153	GGAAAGCG	AGAA	GUUCAG	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UUGAACA GAC CGUCUUC
1157	UAAAGGAA	AGAA	GUCUGU	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	ACAGACC GUC UUCCUUUA
1178	CCCACAUG	AGAA	GAGAAG	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CUUCUCU GUC CAUGUGGG
1246	UCCGAAAG	AGAA	GTUAGC	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GCUAGCU GAU CUUUCGGA
1523	CAGAAAG	AGAA	GGCCUC	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GAGGCUU GCC CUUUUCUG



Table BXVI: Human B7-2 Hairpin Ribozyme and Target Sequences

nt. Position	HP Ribozyme Sequences				Substrate
25	GUUACAGC	AGAA	GAGAAG	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	CUUCUCU GCU GCUGUAAC
28	CCUGUUAC	AGAA	GCAGAG	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	CUUCUCU GCU GUACACAGG
57	CCCCACUC	AGAA	GUGUGU	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	ACACACG GAU GAGUGGGG
162	CACACAG	AGAA	GGAGG	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	CCUUCU GCU CUCUGGUG
175	UUCAGAGG	AGAA	GCACCA	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	UGGUGCU GCU CCUCUGAA
214	CAUGGCAG	AGAA	GCAGUC	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	GACUGCA GAC CUGCCUAG
380	CAGGGUCC	AGAA	GUCGGA	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	UCCGACA GUU GGAUCCUG
408	UGUCCUUG	AGAA	GAAGAU	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	AUCUUA GAU CAAGGACA
480	CAGAUUUC	AGAA	GGUGGA	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	UCCACCA GAU GAUUCUG
575	UAUAGAUG	AGAA	GGUCAA	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	UUGACCU GCU CAUCUAUA
710	AACAGACA	AGAA	GAUGGA	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	UCCAUCA GCU UGUCUGUU
718	GGGAUGA	AGAA	GACAAU	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	CUUGUCU GUU UCAUUCUCC
730	CTUGUAAC	AGAA	GGGAU	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	AUUCUCC GAU GUUACGAG
783	AAGUAUA	AGAA	GGUCU	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	AGACGCG GCU UUUUAUCUU
825	CUGGGGGA	AGAA	GAGGGU	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	ACCUCA GCC UCCUCCAG
835	GGAAUGUG	AGAA	GGGGGA	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	UCCUCCA GAC CACAUUCC
856	GGAAUGUAC	AGAA	GUUAUC	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	GAUUA CAU GUACUUC
896	UAGAUUA	AGAA	GAUAAC	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	GUUUUCU GUC UAAUUCUA
930	AGUUGCGA	AGAA	GCUUCU	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	AGAAGCG GCC UCCGCAACU
987	UUUUCUUG	AGAA	GUUCAC	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	GUGAACA GAC CAAGAAAA
1027	UGGGCUUC	AGAA	GAUCUU	ACCAGAGAAACACACACGUGUGUGGUACAUAUACCUUGUA	AAGAUCU GAU GAAGCCCA

Table BXVII: Mouse B7-2 Hairpin Ribozyme and Target Sequences

nt. Position	HP Ribozyme Sequences				Substrate
10	UCUUAAGC	AGAA	GCUUGC	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	GCAAGCA GAC GCGUAAGA
42	UUGUUCAA	AGAA	GUGCUG	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CAGCAGG GAC UUGAACAA
56	CUACAGGA	AGAA	GGUGU	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	ACAAACCA GAC UCCUGUAG
108	CAUGGUGC	AGAA	GGGGUC	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	GACCCCA GAU GCACCAUG
146	AUCAGCAA	AGAA	GUACACA	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UGUGACA GUC UUGCUGAU
154	CAUCUGAG	AGAA	GCAAGA	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UCUUGCU GAU CUCAGAU
161	GAACAGC	AGAA	GAGUUC	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	GAUCUCA GAU GCUGUUDC
167	UCCACGGA	AGAA	GCAUCU	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	AGAUGCU GUU UCCUGGGA
211	AUGGCCAC	AGAA	GAUUG	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CAUAUCU GCC GUGCCCAU
400	UGUCCUUG	AGAA	GAACAU	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	AUGUUA GAU CAAGGACA
679	AGAUACUG	AGAA	GUUCUG	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CAGNACTU GUU CAGUUAUCU
696	AAGAGAGA	AGAA	GUUGGA	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UCCAACA GCC UCUCUUCU
716	CACACACC	AGAA	GGGAU	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	AUUCGCG GAU GGUGUGUG
737	ACACACAC	AGAA	GUCAUA	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UAUGACC GUU GUGUGUGU
839	GUUACUGA	AGAA	GUAAUC	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	GAUUAAC GCU UCAGUUAAC
874	CAUUGAUG	AGAA	GCAUCA	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UGAUGCU GCU CAUCAUUG
907	GCCUGCUA	AGAA	GAUUCG	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CGAAUCA GCC UAGCAGGC
929	AACUUAAG	AGAA	GUGUUG	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CAACACA GCC UCUAAGUU
1115	UUCCAAUC	AGAA	GAGAAC	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	GUUCUCA GCU GAUUGGAA
1118	GAUUUCCA	AGAA	GCUGAG	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CUACAGCU GAU UGGAUUUC
1133	AUUUUUUC	AGAA	GUAGAA	ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UUUCUACA GUU GAUAUAUU

Table BXVIII : Human CD40 Hairpin Ribozyme and Target Sequences

nt. Position	Hairpin Ribozyme Sequences		Substrate
26	GACCAGGC	AGAA GGACCA ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	UGGUUCCU GCC GCUUGGUC
29	UGAGACCA	AGAA GCAGGA ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	UCCUGCC GCC UGGUCUCA
58	ACUECAGA	AGAA GACGNA ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	UUGGUCU GCC UUGGCAGU
84	GGUCAGCA	AGAA GCCCCA ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	UGGGGCU GCU UGUGAGCC
91	GGACAGCG	AGAA GCAAGC ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	GCTUUGU GAC CGCUGUCC
95	GGUUGGAC	AGAA GUCAGC ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	GCUGAAC GCU GUCCAUCG
98	UCUGGAUG	AGAA GCGGUC ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	GACCGCU GUC CAUCCAGA
159	GCACAAAG	AGAA GCACUG ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	CAGUGCU GUU CUIUGUGC
414	CGAGCAUG	AGAA GUGCAG ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	CUGCAAC GCU CAUGCUUG
429	GACCCCAA	AGAA GGGGGA ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	UGGCCCG GCU UUGGGGUC
445	CUGUAGCA	AGAA GCUUGA ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	UCAAGCA GAU UGCUACAG
483	GCGGACUG	AGAA GGGCUC ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	GAGCCCU GCC CAGUCGCG
488	AGAGAGCC	AGAA GGGCAG ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	CUGCCCA GUC GGUUUCUU
492	GGAGAGAG	AGAA GACUUG ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	CCAGUCG GCU UCUUCUCC
515	UUUUGGAA	AGAA GAUGAC ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	GUCAUCU GCU UUCGAAAA
593	CAGACNAC	AGAA GUCUUG ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	CAAGACU GAU GUUGUUG
619	GCGCUCUC	AGAA GAUCCU ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	AGGAUUG GCU GAGAGCCC
661	GGUUGGCA	AGAA GGAUCC ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	GGAUCCU GUU UGCGCAUCC
764	GGAGAUUC	AGAA GGAANA ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	UUUUCOC GAC GAUCUUCG
788	ACUGGAGC	AGAA GUGUUG ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	CAACACU GCU GCUCACAGU
791	UGCACUGG	AGAA GCAGUG ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	CACUGCU GCU CCAGUGCA
924	CUUGGCC	AGAA GCTUGU ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	ACAGGCA GUU GGGCAGAG
946	CTUGCAGC	AGAA GCACCA ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	UGGUGCU GCU GCUCCAGG
949	ACCCUUGC	AGAA GCAGCA ACCAGAGAAACACACAGGUUGUGGUACAUAUACCTUGGUA	UGCUGCU GCU GCAGGGGU

Table BXIX: Mouse CD40 Hairpin Ribozyme and Substrate Sequences

nt. Position	HP Ribozyme Sequences	Substrate
25	GCGCGCAC AGAA GAGGCA ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	UGCCUUG GCU GUGCGCGC
45	UGUCAACA AGAA GCGCCA ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	UGGGGCU GCU UGUUGACA
59	CTUAGAUG AGAA GCTGUC ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	GACAGCG GUC CAUCUAGG
144	GCTUGUCA AGAA GCTUCC ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	GGAAGCC GAC UGACAAGC
164	UUCUCAAG AGAA GUGCAG ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	CTGCACA GCU CUUGAGAA
212	UUCCACTUG AGAA GAGAAU ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	AUUTUCA GCC CAGUGGAA
311	CAGGUACA AGAA GUGUCU ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	AGACACU GUC UGUACCTUG
431	GGAUGACA AGAA GUAUCA ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	UGAUACC GUC UGUACUCC
444	GCCGACTUG AGAA GGGGUG ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	CAUCCCU GCC CAGUCGGC
449	AAGNAGCC AGAA GGGCAG ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	CUGCCCA GUC GGTUUCUU
453	GGAGNAGA AGAA GATUGG ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	CCAGUUG GCU UCUUUCUC
550	UGACAUUA AGAA GATUCG ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	CGAGUCA GAC UAAUGUCA
580	GCGCUCCG AGAA GGGGACU ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	AGUCCCG GAU GCGAGCCC
592	GAAUGACC AGAA GGGCTUC ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	GAGCCCU GCU GGUCAUUC
605	CCCAUCAC AGAA GGAUUG ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	CAUUCUU GUC GUGAUGGG
701	UGCGGUCG AGAA GCAGGG ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	CCCUGGG GCU CGACGGCA
752	ACUGGAGC AGAA GUGTUA ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	UAAACCC GCU GCUCCAGU
755	UGCACUGG AGAA GCGGUG ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	CACCGCU GCU CCAGUGCA
787	GUGUGACA AGNA GACACC ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	GGUGUCA GCC UGUCACAC
890	CUUCUAAA AGNA GUUCCA ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	UGGAACU GCU UUUGGAGG
909	GGUCACCA AGAA GCCAUC ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	GAUGGCU GCU UGUCGACC
916	UUCAAAAG AGAA GCAAGC ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	GCTUGCU GAC CUUUUGAA
975	UGACAGGG AGAA GCGAUG ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	CAUGCCU GCC CCCUGUCA
1137	CGAGCACA AGAA GCGGGC ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	GCCCGCA GCU UGUUGCTUG
1276	GUUUUAAA AGAA GUUUUU ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	AGAAACA GCU UUUUAAAC
1334	CGGGUUUG AGAA GCAAGC ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	GCTUUGCU GCC CAACCCCG
1352	GGAUCAAA AGAA GGUAAAC ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	GUUACCU GAU UUUUAUCC
1512	AAACCCAG AGAA GAUUAA ACCAGAGAAACACACGUGUGGUACAUAUACCTUGGUA	UUAAUCC GCC CUGGGUUU

Table CII: 2.5  $\mu$ mol RNA Synthesis Cycle

Reagent	Equivalents	Amount	Wait Time*
Phosphoramidites	6.5	163 $\mu$ L	2.5
S-Ethyl Tetrazole	23.8	238 $\mu$ L	2.5
Acetic Anhydride	100	233 $\mu$ L	5 sec
N-Methyl Imidazole	186	233 $\mu$ L	5 sec
TCA	83.2	1.73 mL	21 sec
Iodine	8.0	1.18 mL	45 sec
Acetonitrile	NA	6.67 mL	NA

\* Wait time does not include contact time during delivery.

Table EVII: Deprotection of a 36 mer all ribo oligo using 70% ethylamine in aqueous. The data are as follows upon HPLC reprocessing:

Sample	OD's	% Full Length Product (FLP)	% frontside	%backside
MA 10'@65°	0.984	14.5073	71.6740	13.8186
MA 10'@65°	1.125	18.9269	67.8006	13.2725
EA rt 10'	0.925	16.5804	66.8186	16.6010
EA rt 10'	0.920	15.7421	67.5794	16.6785
EA rt 30'	0.971	17.4694	67.6782	14.8525
EA rt 30'	0.794	15.7587	69.8084	14.4329
EA 40° 10'	0.819	18.0827	66.4937	15.4236
EA 40° 10'	0.986	17.5763	66.7865	15.6372
EA 40° 15'	0.877	18.7963	67.0064	14.1999
EA 40° 15'	0.911	18.7808	70.7306	10.4885
EA 55° 10'	1.001	17.8810	66.4703	15.6487
EA 55° 10'	1.023	19.1069	68.6706	12.2225

Claims

1. An enzymatic nucleic acid having a hammerhead motif, wherein said nucleic acid comprises of at least five ribose residues, and wherein  
5 said nucleic acid comprises a 2'-C-allyl modification at position No. 4 of said nucleic acid, and wherein said nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'- end modification.
2. The enzymatic nucleic acid of claim 1, wherein said nucleic acid  
10 comprises a 3'-3' linked inverted ribose moiety at said 3' end.
3. An enzymatic nucleic acid having a hammerhead motif, wherein said nucleic acid comprises of at least five ribose residues, and wherein said nucleic acid comprises a 2'-amino modification at position No. 4 and/or at position No. 7 of said nucleic acid, wherein said nucleic acid  
15 comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'-3' linked inverted ribose or thymidine moiety at its 3' end.
4. An enzymatic nucleic acid having a hammerhead motif, wherein said nucleic acid comprises of at least five ribose residues, and wherein  
20 said nucleic acid comprises a non-nucleotide substitution at position No. 4 and/or at position No. 7 of said nucleic acid molecule, wherein said nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'-3' linked inverted ribose or thymidine moiety at its 3' end.
- 25 5. An enzymatic nucleic acid which cleaves target mRNA having a sequence selected from SEQ. ID. NOS. 34, 35, 57, 125, 126, 127, 128, 129, 140, 162, 170, 179, 188, 223, 224, 236, 245, 246, 256, 259, 260, and 281, wherein said nucleic acid comprises of at least five ribose residues, and wherein said nucleic acid comprises a 6-methyl  
30 uridine substitution at position No. 4 and/or at position No. 7 of said nucleic acid molecule, wherein said nucleic acid comprises at least

ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'-3' linked inverted ribose or thymidine moiety at its 3' end.

- 5 6. The enzymatic nucleic acid which cleaves target mRNA having a sequence selected from SEQ. ID. NOS. 34, 35, 57, 125, 126, 127, 128, 129, 140, 162, 170, 179, 188, 223, 224, 236, 245, 246, 256, 259, 260, and 281, wherein said nucleic acid comprises of at least five ribose residues, wherein said nucleic acid comprises a 2'-C-allyl modification at position No. 4 of the said nucleic acid, wherein said  
10 nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 2'-3' linked inverted ribose or thymidine moiety at its 3' end.
- 15 7. The enzymatic nucleic acid of any one of claims 1-6, wherein said nucleic acid comprises phosphorothioate linkages at least three of the seven 5' terminal nucleotides.
8. Nucleic acid molecule which blocks synthesis and/or expression of an mRNA encoding B7-1, B7-2, B7-3 and/or CD40.
9. The nucleic acid of claim 8, wherein said molecule is an enzymatic nucleic acid molecule.
- 20 10. The nucleic acid molecule of claim 9, wherein, the binding arms of said enzymatic nucleic acid contain sequences complementary to the nucleotide base sequences in any one of Tables BII, BIV, BVI, BVIII, BX, BXII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX.
- 25 11. The nucleic acid molecule of claims 9 or 10, wherein said nucleic acid molecule is in a hammerhead motif.
12. The enzymatic nucleic acid molecule of claim 9 or 10, wherein said nucleic acid molecule is in a hairpin, hepatitis Delta virus, group I intron, VS nucleic acid or RNaseP nucleic acid motif.



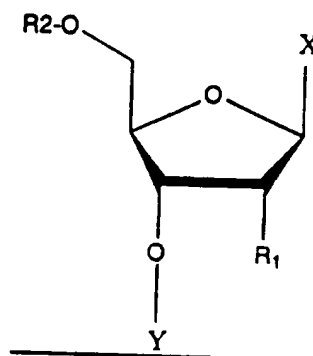
13. The enzymatic nucleic acid molecule of any of claims 9 or 10, wherein said ribozyme comprises between 12 and 100 bases complementary to the RNA of said region.
- 5 14. The enzymatic nucleic acid of claim 13, wherein said ribozyme comprises between 14 and 24 bases complementary to the RNA of said region.
- 15 15. Enzymatic nucleic acid molecule consisting essentially of any ribozyme sequence selected from those shown in Tables BIII, BV, BVI, BVII, BIX, BXI, BXIII, BXIV, BXV, BXVI, BXVII, BXVIII.
- 10 16. A mammalian cell including an enzymatic nucleic acid molecule of any of claims 8 or 9.
17. The cell of claim 16, wherein said cell is a human cell.
- 15 18. An expression vector comprising nucleic acid encoding the enzymatic nucleic acid molecule of any of claims 9 or 10, in a manner which allows expression and/or delivery of that enzymatic RNA molecule within a mammalian cell.
19. A mammalian cell including an expression vector of claim 18.
- 20 20. The cell of claim 19, wherein said cell is a human cell.
21. A method for treatment of a patient having a condition associated with the level of B7-1, B7-2, B7-3 and/or CD40, wherein the patient, tissue donor or population of corresponding cells is administered a therapeutically effective amount of an enzymatic nucleic acid molecule of claims 8, 9 or 10.
- 25 22. A method for treatment of a condition related to the level of B7-1, B7-2, B7-3 and/or CD40 activity by administering to a patient an expression vector of claim 21.
23. The method of claims 21 or 22, wherein said patient is a human.

24. A method for inducing tolerance in a recipient to alloantigen of a donor comprising treating antigen presenting cells from a donor with nucleic acid of claim 8 or 9, and infusion of said treated antigen presenting cells into said recipient.
- 5 25. A method for enhancing graft tolerance comprising contacting a nucleic acid of claims 8 or 9 with cells of said graft prior to transplantation.
26. A method for treatment of an autoimmune disease, comprising contacting an antigen presenting cell of a patient with a nucleic acid of claims 8 or 9.
- 10 27. The method of claim 26, wherein said cells are contacted *ex vivo* with said nucleic acid.
28. The method of claim 26, wherein said cells are contacted with autoantigen characteristic of said disease.
- 15 29. The method of claim 28, wherein said cells are reinfused into said patient.
30. Enzymatic nucleic acid having at least one modified base substitution, wherein said base substitution is selected from a group comprising pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyluracil, dihydrouracil, naphthyl, 6-methyl-uracil and aminophenyl.
- 20 31. The enzymatic nucleic acid of any of claim 30, wherein said nucleic acid has a hammerhead motif.
32. Mammalian cell comprising an enzymatic nucleic acid molecule of and of claims 30-31.
- 25 33. The enzymatic nucleic acid of claim 31, wherein said nucleic acid includes said modified base substitutions at position 4 or at position 7.
34. The ribozyme of claim 33, wherein said substitution is 6-methyl uracil.
35. The ribozyme of claim 33, wherein said substitution is pyridin-4-one.

36. The ribozyme of claim 33, wherein said substitution is phenyl.
37. The ribozyme of claim 33, wherein said substitution is pyridin-2-one.
38. The ribozyme of claim 33, wherein said substitution is pseudouracil.
- 5 39. The ribozyme of claim 33, wherein said substitution is 2, 4, 6-trimethoxy benzene.
40. The ribozyme of claim 33, wherein said substitution is dihydrouracil.
41. The ribozyme of claim 33, wherein said substitution is 3-methyluracil.
42. The ribozyme of claim 33, wherein said substitution is naphthyl.
43. The ribozyme of claim 33, wherein said substitution is aminophenyl.
- 10 44. 2'-deoxy-2'-alkylnucleoside.
45. 2'-deoxy-2'-alkylnucleotide.
46. Oligonucleotide comprising one or more 2'-deoxy-2'-alkylnucleotides.
47. Enzymatic nucleic acid comprising a 2'-deoxy-2'-alkylnucleotide.
- 15 48. Method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, comprising the step of forming said enzymatic molecule with at least one nucleotide having at its 2'-position an alkyl group.
49. 2'-deoxy-2'-alkylnucleotide triphosphate.
- 20 50. Method for synthesis of a 2'-C-allyl derivative from a 5'-O-DMT-3'-O-TBDMS-base comprising the steps of:
- (a) phenoxyltricarboxylation of 5'-O-DMT-3'-O-TBDMS-base to yield a thioester, replacing a 2' hydroxyl group with a phenoxythiocarbonyl group, and

(b) Heck acylation of said thioester to form a 2'-C-allyl derivative in which said 2'-phenoxythiocarbonyl group is replaced with said 2'-C-alkyl group to yield said 2'-C-allyl derivative.

51. A compound having the formula:



wherein, R1 represents 2'-O-alkylthioalkyl or 2'-C-alkylthioalkyl; X represents a base or H; Y represents a phosphorus-containing group; and R2 represents O, DMT or a phosphorus-containing group.

52. Oligonucleotide comprising one or more compounds of claim 51.
53. Enzymatic nucleic acid comprising a compound of claim 51.
54. The compound of claim 51, wherein said compound is in the form of a triphosphate.
55. Enzymatic nucleic acid of claim 53 wherein said nucleic acid is in a hammerhead motif.
56. Enzymatic nucleic acid of claim 53, wherein said nucleic acid is in a hairpin, hepatitis delta virus, group I intron, VS RNA or RNase P RNA motif.
57. Enzymatic nucleic acid of claim 55, wherein said hammerhead ribozyme has positions 4 and/or 7 substituted with 2'-O-methylthiomethyl.

58. Enzymatic nucleic acid of claim 55 or 57, wherein one monomer in stem II of said hammerhead is substituted with at least one 2'-O-methylthiomethyl.
59. Enzymatic nucleic acid of claim 55 or 56, wherein said nucleic acid is substituted at one or more positions with 2'-O-methylthiophenyl.
60. A mammalian cell comprising a compound of any one of the claims 51-59.
61. The cell of claim 60, wherein said cell is a human cell.
62. Method for producing an enzymatic nucleic acid molecule having activity to cleave an RNA or single-stranded DNA molecule, comprising the step of forming said enzymatic molecule with at least one position having at its 2'-position an 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl group.
64. Hammerhead ribozyme having a non-nucleotide in the catalytic core in a site selected from the group consisting of the normally occurring uracil at position 4 and 7.
65. Hammerhead ribozyme having a stem II and a loop II, wherein said loop II comprises a non-nucleotide.
66. Hammerhead ribozyme having a non-nucleotide at its 3' end.
67. A mammalian cell comprising an enzymatic nucleic acid molecule of any one of the claims 64-67.
68. The cell of claim 67, wherein said cell is a human cell.
69. Method of synthesis of abasic ribonucleoside mimetics described in figure 58.
70. A method for the deprotection of RNA comprising the step of providing aqueous ethylamine (EA) at between 25°C - 60°C for 5 to 30 minutes to remove any exocyclic amino protecting groups from protected RNA.

71. The method of claim 70 wherein, said ethylamine is provided at 40°C for 10 minutes.
72. The method of claim 70 wherein, said ethylamine is provided at 55°C for 10 minutes.
- 5 73. The method of claim 70, further comprising deprotection of RNA alkylsilyl protecting groups comprising, contacting said groups with anhydrous triethylamine-hydrogen fluoride (aHF•TEA) trimethylamine or diisopropylethylamine at between 60 °C-70 °C for 0.25-24 h.
- 10 74. The method of any one of claims 70-73 wherein, said RNA is an enzymatic RNA.
75. Method for synthesis of an enzymatic nucleic acid, comprising the steps of:
- 15 providing a 3' and a 5' portion of said enzymatic nucleic acid having independent chemically reactive groups at the 5' and 3' positions, respectively, under conditions in which a covalent bond is formed between said 3' and 5' portions by said chemically reactive groups, said bond being selected from the group consisting of, disulfide, morpholino, amide, ether, thioether, amine, a double bond, sulfonamide, ester, carbonate, hydrazone, said bond not being a
- 20 natural bond formed between a 5' phosphate group and a 3' hydroxyl group.
76. The method of claim 75, wherein said nucleic acid has a hammerhead motif and said 3' and 5' positions each have said chemically reactive groups in or immediately adjacent to the stem II region.
- 25 77. The method of claim 75, wherein one said chemically reactive group is  $(CH_2)_nSH$  and the other chemically reactive group is  $(CH_2)_nSH$ , wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 30 78. The method of claim 75, wherein one said chemically reactive group is  $(CH_2)_nNH_2$  and the other chemically reactive group is ribose, wherein

each  $n$  independently is an integer from 0 to 10 inclusive and may be the same or different.

- 5 79. The method of claim 75, wherein one said chemically reactive group is  $(\text{CH}_2)_n\text{NH}_2$  and the other chemically reactive group is  $\text{COOH}$ , wherein each  $n$  independently is an integer from 0 to 10 inclusive and may be the same or different.
- 10 80. The method of claim 75, wherein one said chemically reactive group is  $(\text{CH}_2)_n\text{X}$  and the other chemically reactive group is  $(\text{CH}_2)_n\text{OH}$  or  $(\text{CH}_2)_n\text{SH}$ ; wherein each  $n$  independently is an integer from 0 to 10 inclusive and may be the same or different; X is halogen.
- 15 81. The method of claim 75, wherein one said chemically reactive group is  $(\text{CH}_2)_n\text{NH}_2$  and the other chemically reactive group is  $\text{CHO}$ , wherein each  $n$  independently is an integer from 0 to 10 inclusive and may be the same or different.
- 20 82. The method of claim 75, wherein one said chemically reactive group is  $(\text{CH}_2)_n\text{PPh}_3$  and the other chemically reactive group is  $\text{CHO}$ , wherein each  $n$  independently is an integer from 0 to 10 inclusive and may be the same or different.
- 25 83. The method of claim 75, wherein one said chemically reactive group is  $(\text{CH}_2)_n\text{NH}_2$  and the other chemically reactive group is  $(\text{CH}_2)_n\text{SO}_2\text{Cl}$ , wherein each  $n$  independently is an integer from 0 to 10 inclusive and may be the same or different.
- 30 84. The method of claim 75, wherein one said chemically reactive group is  $(\text{CH}_2)_n\text{OH}$  and the other chemically reactive group is  $\text{COOH}$ , wherein each  $n$  independently is an integer from 0 to 10 inclusive and may be the same or different.
85. The method of claim 75, wherein one said chemically reactive group is  $(\text{CH}_2)_n\text{COH}$  and the other chemically reactive group is  $(\text{CH}_2)_n\text{NH}_2$ , wherein each  $n$  independently is an integer from 0 to 10 inclusive and may be the same or different.

86. The method of claim 75, wherein one said chemically reactive group is  $(CH_2)_nCOX$  and the other chemically reactive group is  $(CH_2)_nOH$ , wherein each  $n$  independently is an integer from 0 to 10 inclusive and may be the same or different.
- 5 87. The method of claim 78, wherein said conditions include provision of  $NaIO_4$  in contact with said ribose, and subsequent provision of  $NaBH_4$  or  $NaCNBH_3$ .
88. The method of claim 79, wherein said conditions include provision of a coupling reagent.
- 10 89. A mixture comprising 5' and 3' portions of an enzymatic nucleic acid having a 3' and 5' chemically reactive group respectively selected from the group consisting of  $(CH_2)_nSH$ ,  $(CH_2)_nNH_2$ , ribose,  $COOH$ ,  $(CH_2)_nX$ ,  $(CH_2)_nPPh_3$ ,  $CHO$ ,  $(CH_2)_nSO_2Cl$ ,  $(CH_2)_nCOX$ ,  $(CH_2)_nX$ ,  $(CH_2)_nOH$ ,  $(CH_2)_nCOH$ , and  $(CH_2)_nSH$ ; wherein each  $n$  independently is an integer from 0 to 10 inclusive and may be the same or different and  $X$  is halogen.
- 15 90. The method of claim 75, wherein one said chemically reactive group is linking group-SH and the other chemically reactive group is linking group-SH, wherein each linking group may be the same or different.
- 20 91. The method of claim 75, wherein one said chemically reactive group is linking group- $NH_2$  and the other chemically reactive group is ribose.
92. The method of claim 75, wherein one said chemically reactive group is linking group- $NH_2$  and the other chemically reactive group is  $COOH$ .
- 25 93. The method of claim 75, wherein one said chemically reactive group is linking group- $X$  and the other chemically reactive group is linking group-OH or linking group-SH; wherein each linking group may be the same or different;  $X$  is halogen.
94. The method of claim 75, wherein one said chemically reactive group is linking group- $NH_2$  and the other chemically reactive group is  $CHO$ .



95. The method of claim 75, wherein one said chemically reactive group is linking group-PPh<sub>3</sub> and the other chemically reactive group is CHO.
96. The method of claim 75, wherein one said chemically reactive group is linking group-NH<sub>2</sub> and the other chemically reactive group is linking group-SO<sub>2</sub>Cl, wherein each linking group may be the same or different.
97. The method of claim 75, wherein one said chemically reactive group is linking group-OH and the other chemically reactive group is COOH.
98. The method of claim 75, wherein one said chemically reactive group is linking group-COH and the other chemically reactive group is linking group-NH<sub>2</sub>, wherein each linking group may be the same or different.
99. The method of claim 75, wherein one said chemically reactive group is linking group-COX and the other chemically reactive group is linking group-OH, wherein each linking group may be the same or different.
100. The method of claim 91, wherein said conditions include provision of NaIO<sub>4</sub> in contact with said ribose, and subsequent provision of NaBH<sub>4</sub> or NaCNBH<sub>3</sub>.
101. The method of claim 100, wherein said conditions include provision of a coupling reagent.
102. A mixture comprising 5' and 3' portions of an enzymatic nucleic acid having a 3' and 5' chemically reactive group respectively selected from the group consisting of linking group-SH, linking group-NH<sub>2</sub>, ribose, COOH, linking group-X, linking group-PPh<sub>3</sub>, CHO, linking group-SO<sub>2</sub>Cl, linking group-COX, linking group-X, linking group-OH, linking group-COH, and linking group-SH; wherein each linking group may be the same or different and X is halogen.
103. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said

stem comprises at least 8 base pairs wherein said molecule is transcribed by a RNA polymerase II promoter system.

- 5 104. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs, wherein said molecule is transcribed by a U6 small nuclear RNA promoter system.
- 10 105. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs, wherein said molecule is transcribed by an adenovirus VA1 RNA promoter system.
- 15 106. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs, wherein said molecule is a chimeric adenovirus VA1 RNA.
- 20 107. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs, wherein said intramolecular stem is separated from said desired RNA by a spacer sequence.
- 25 108. The RNA molecule of claim 107, wherein said spacer sequence is about 5-50 nucleotides.

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FIG. 1.

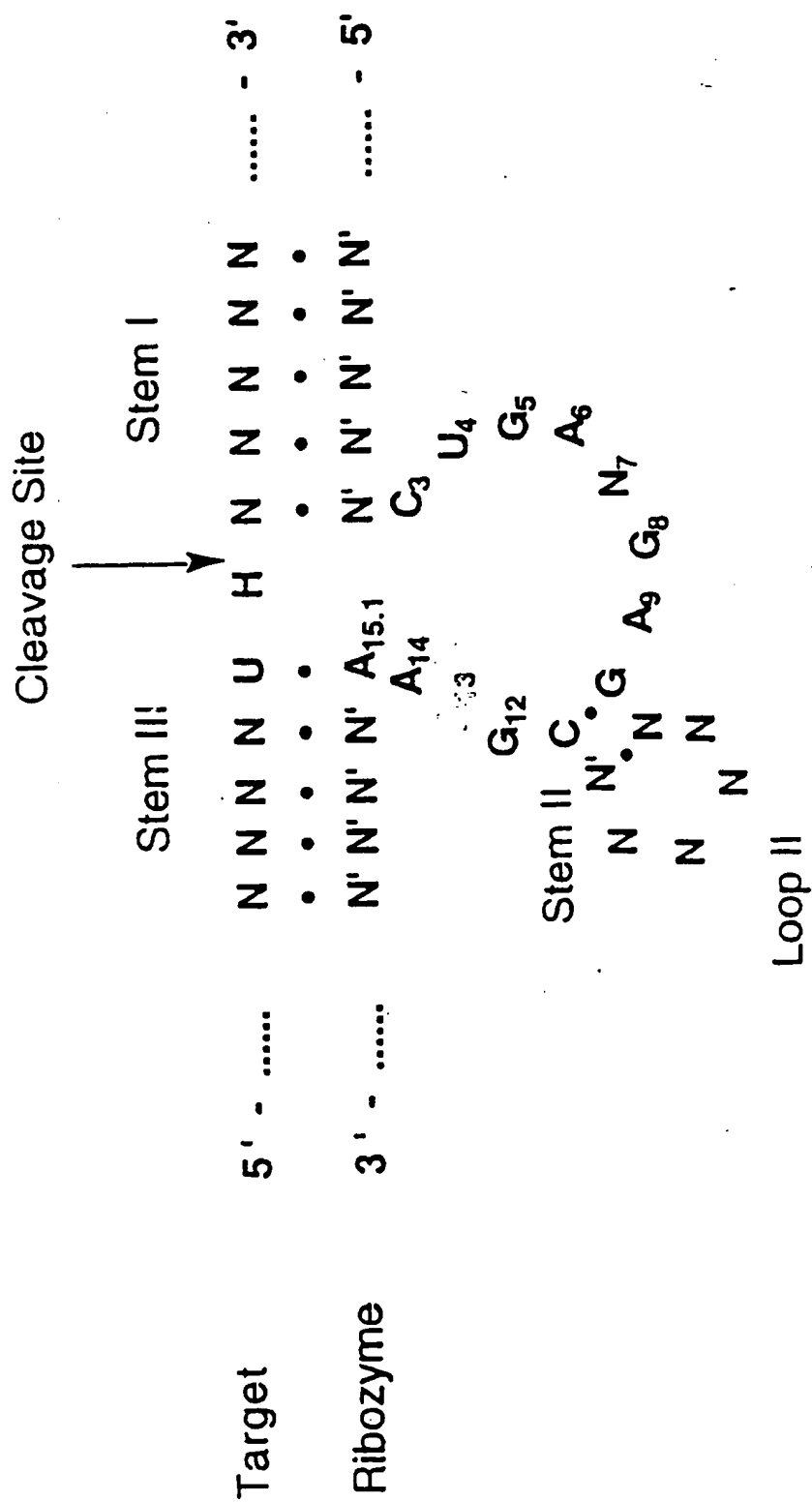


FIG. 2a.

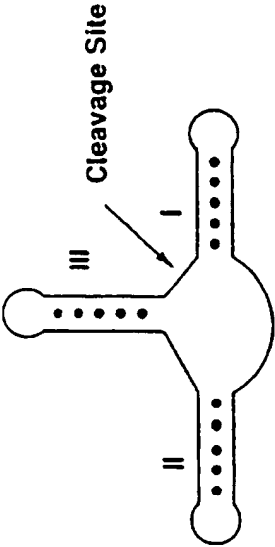


FIG. 2b.

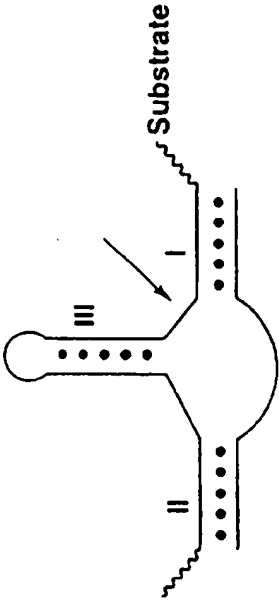


FIG. 2c.

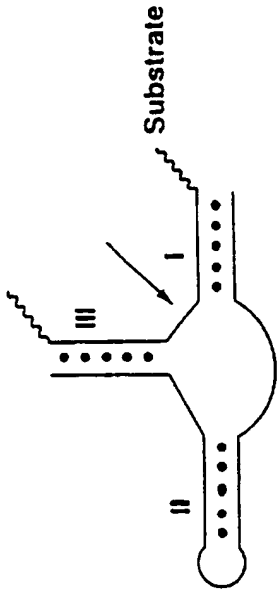
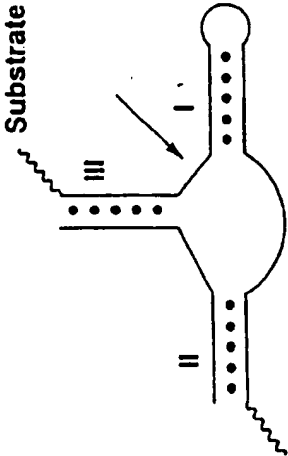
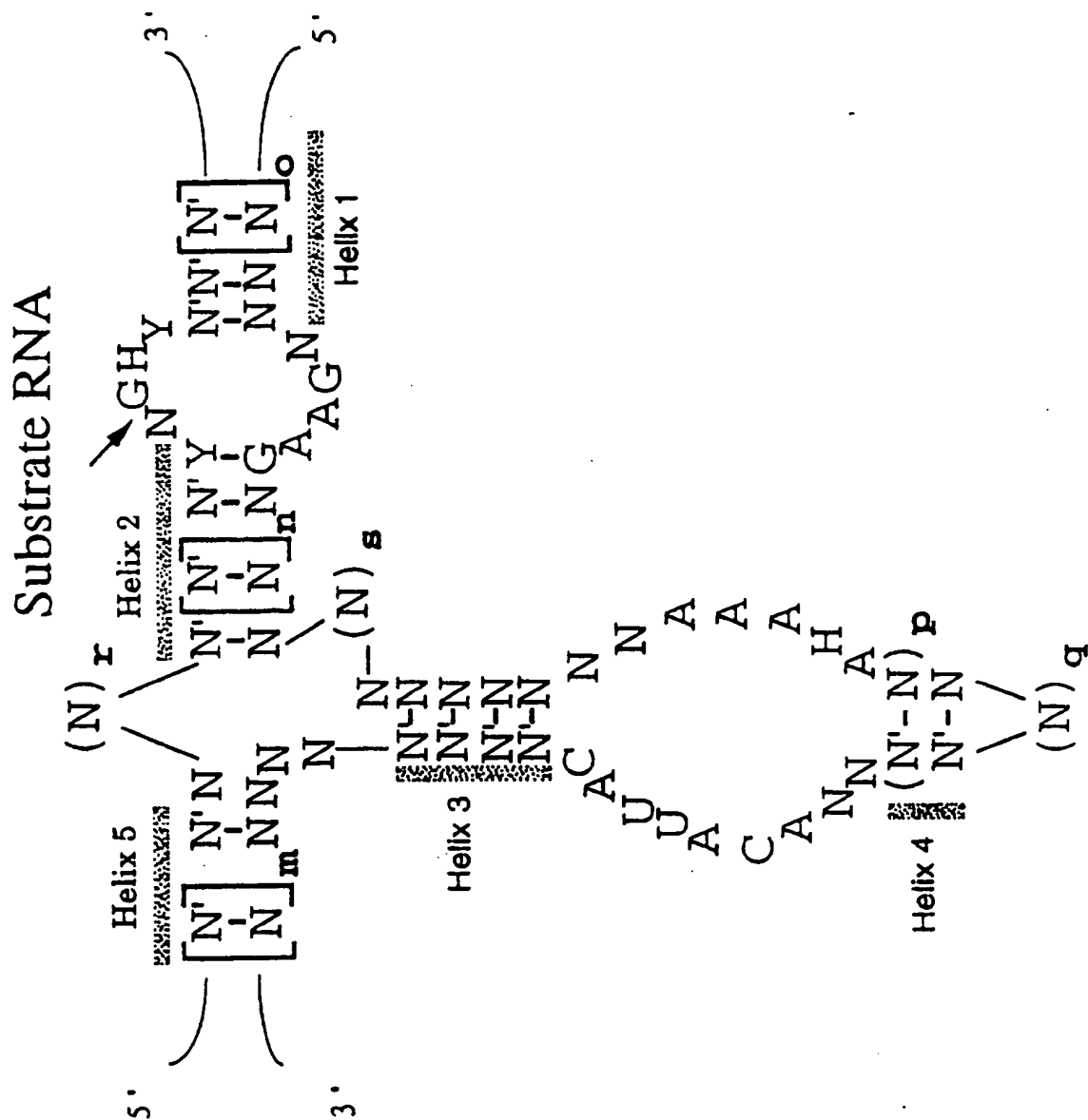


FIG. 2d.



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FIG. 3.







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FIG. 6b.

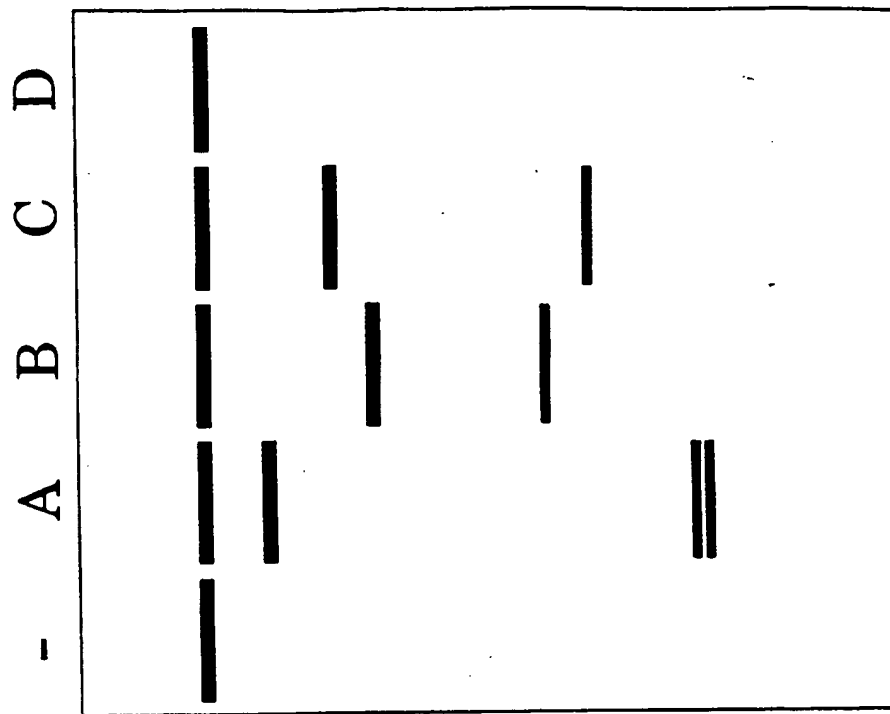
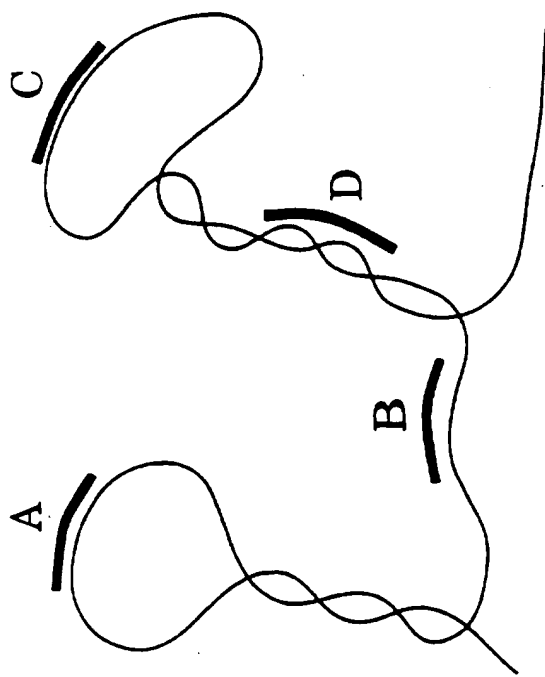


FIG. 6a.



Body-labeled transcript  
(not purified)

• DNA oligo  
(10 nM, 100 nM and 1000 nM)

RNAse H  
(0.08 - 1.0 u/μl)

37°C, 10 min



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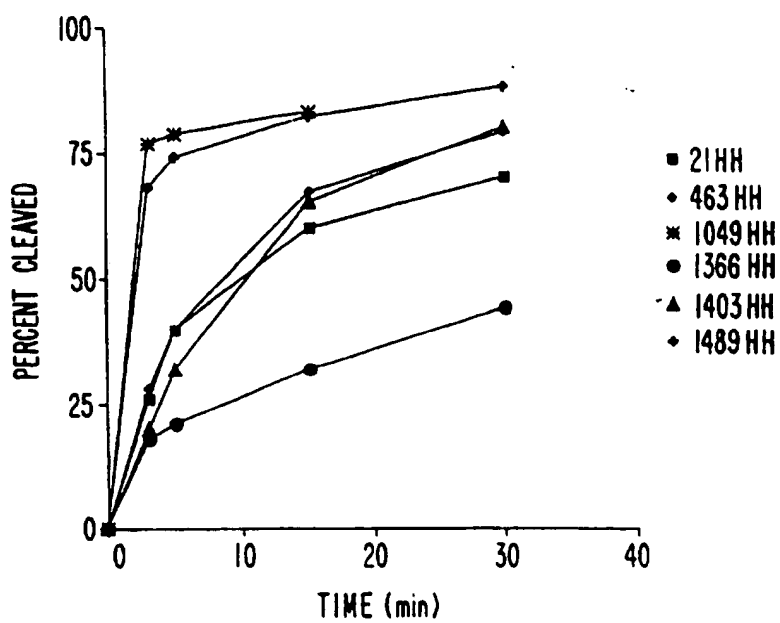


FIG. 7.

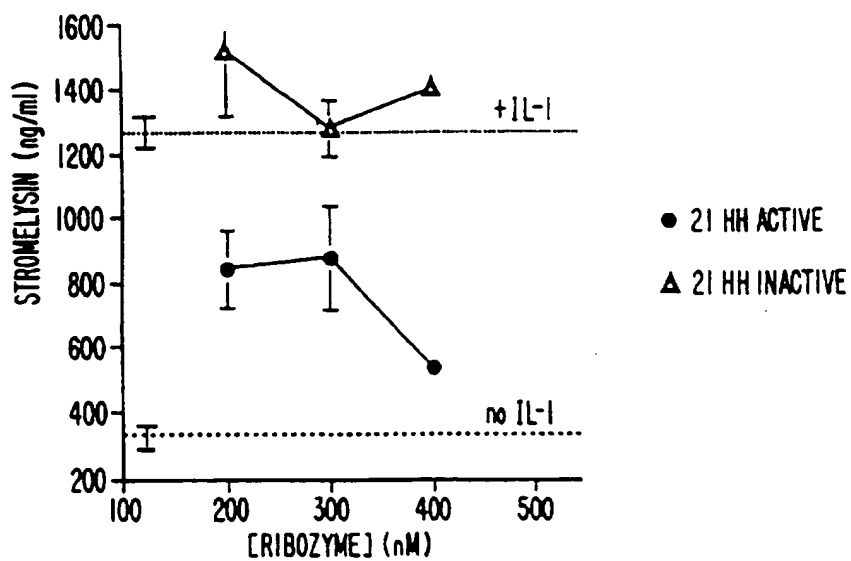


FIG. 8.

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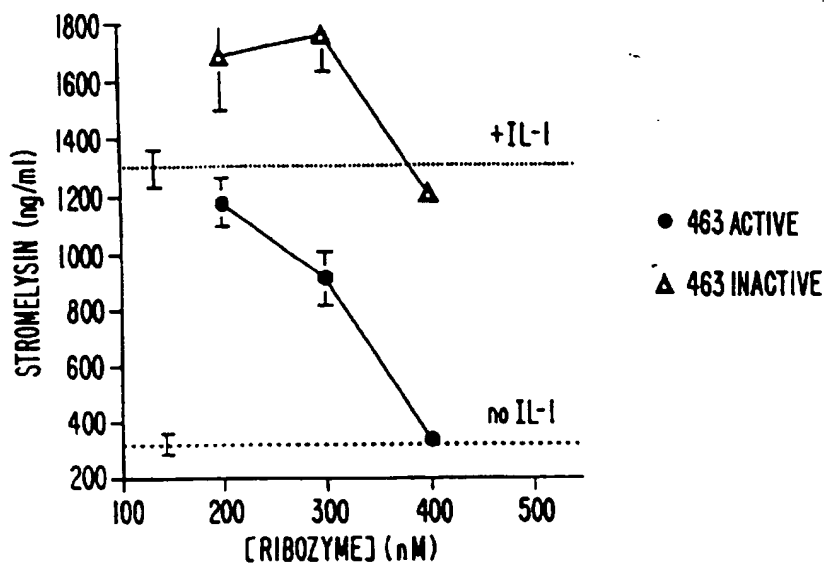


FIG. 9.

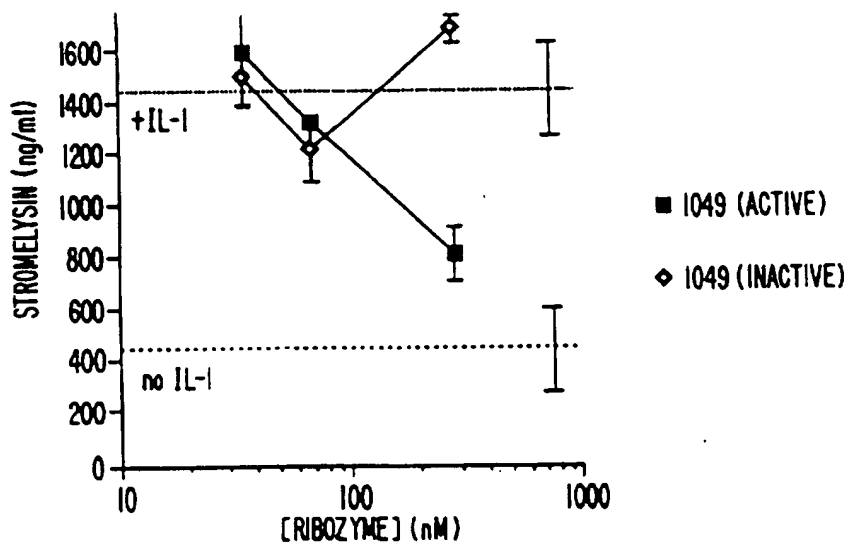


FIG. 10.

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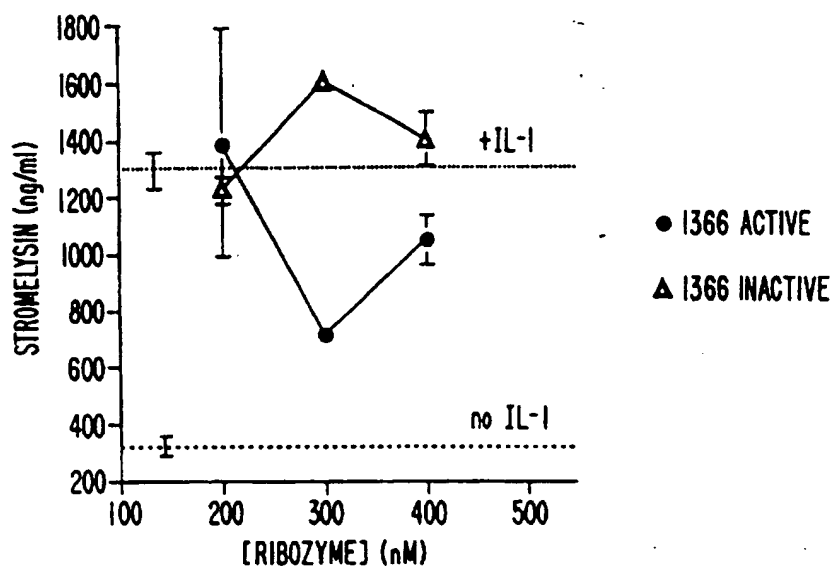


FIG. 11.

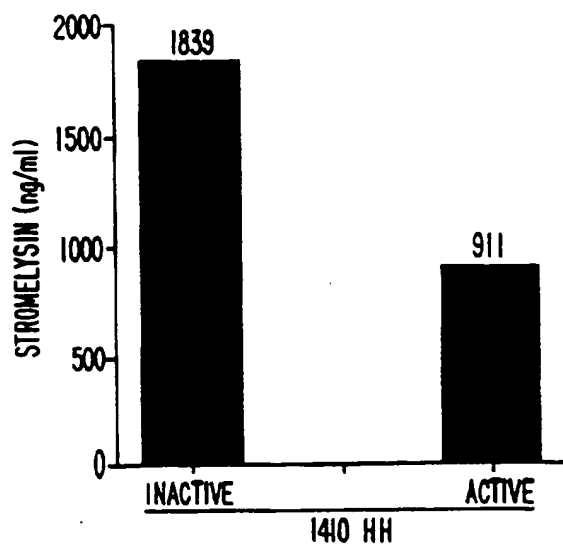


FIG. 12.

FIG. 14.

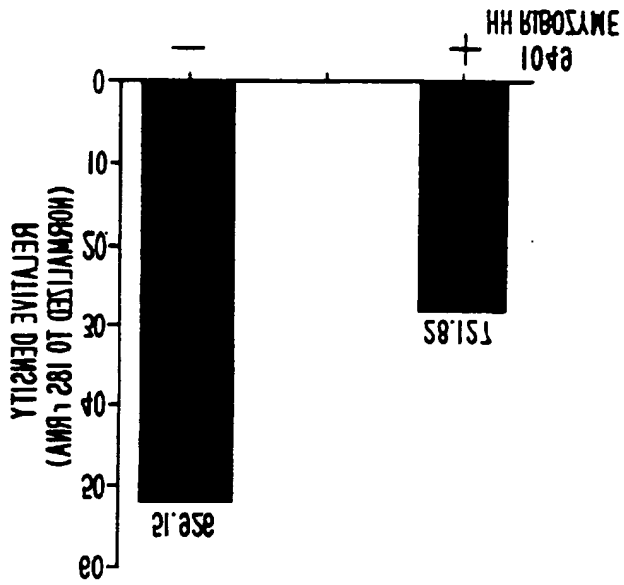
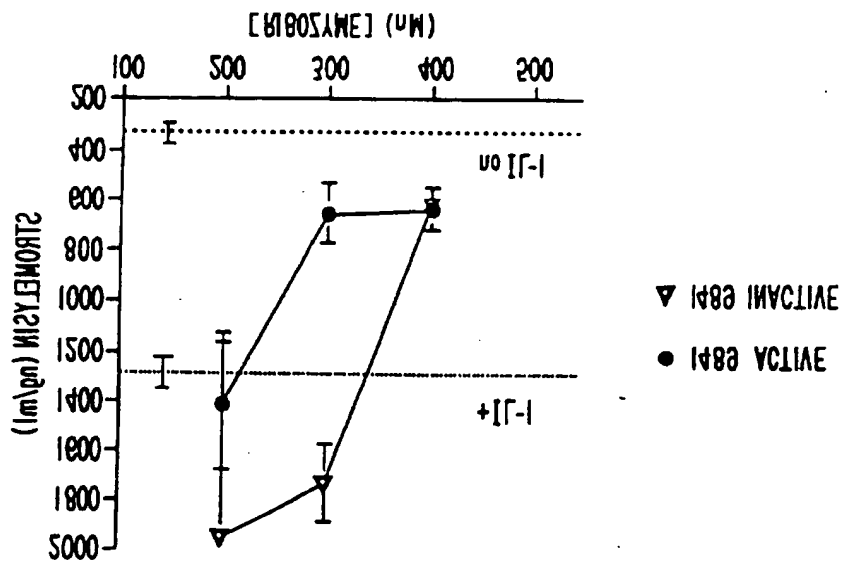


FIG. 13.



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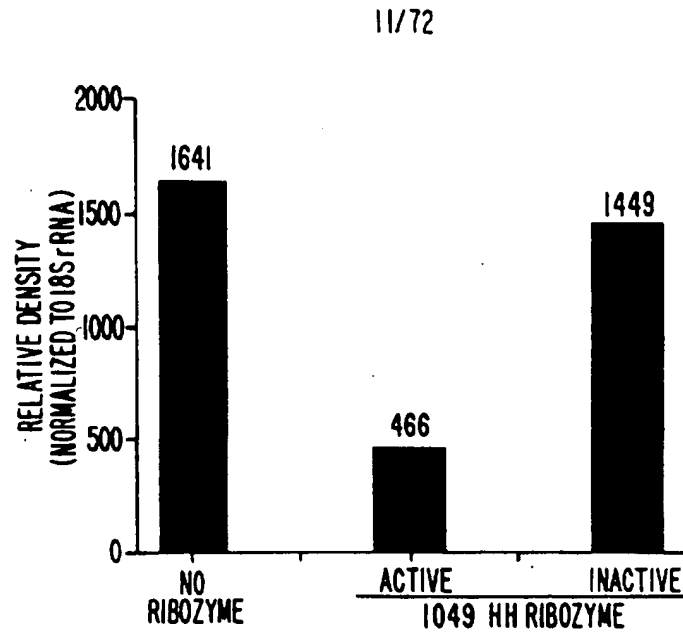


FIG. 15.

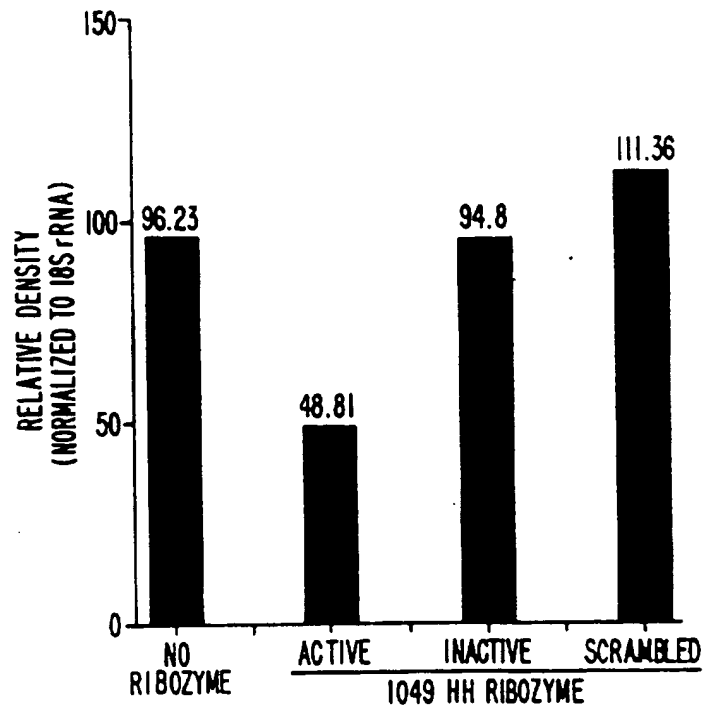
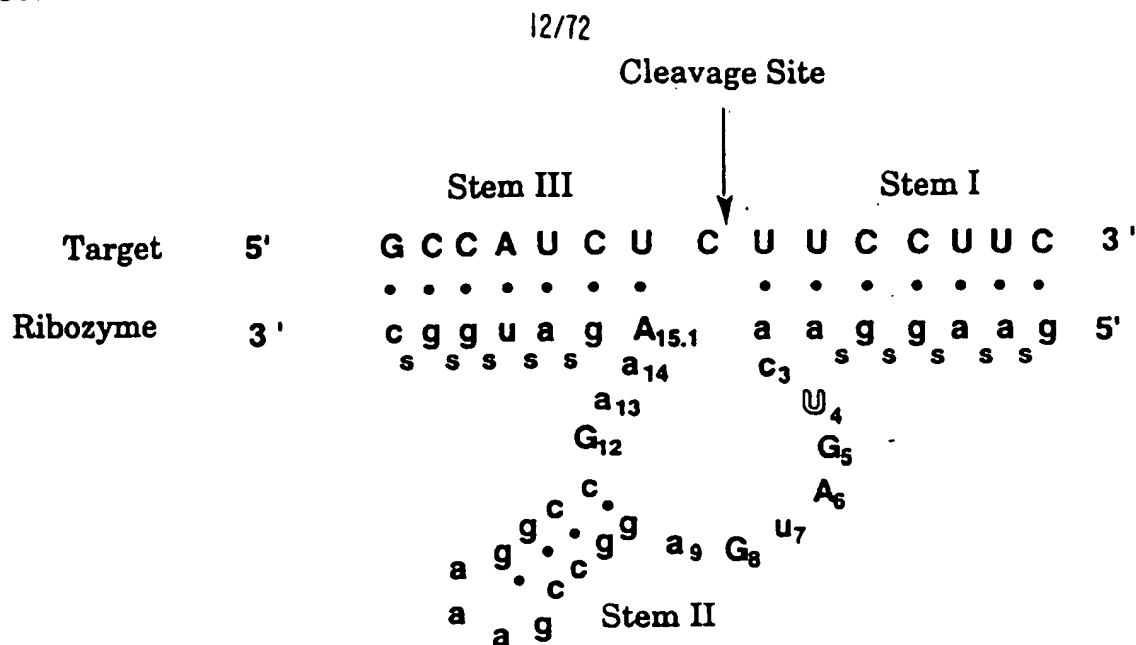


FIG. 16.



Upper case= ribonucleotides  
 Lower case= 2'-O-methyl nucleotides  
 U = 2'-C-Allyl modification  
 s = phosphorothioate linkages

FIG. 17a.

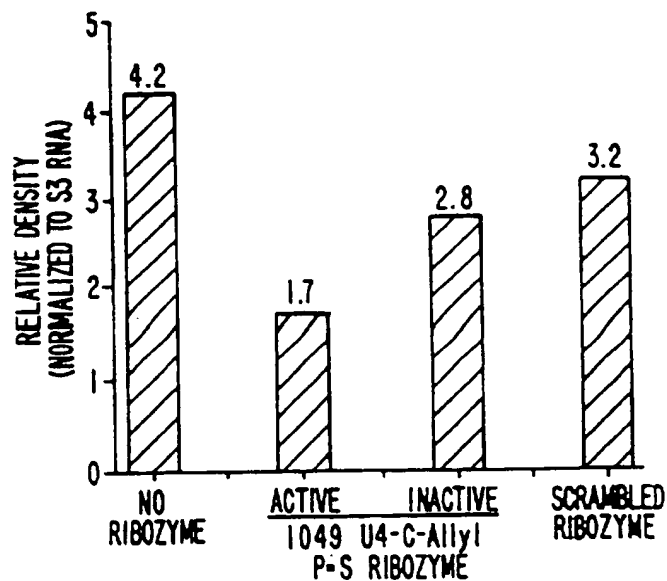


FIG. 17b.

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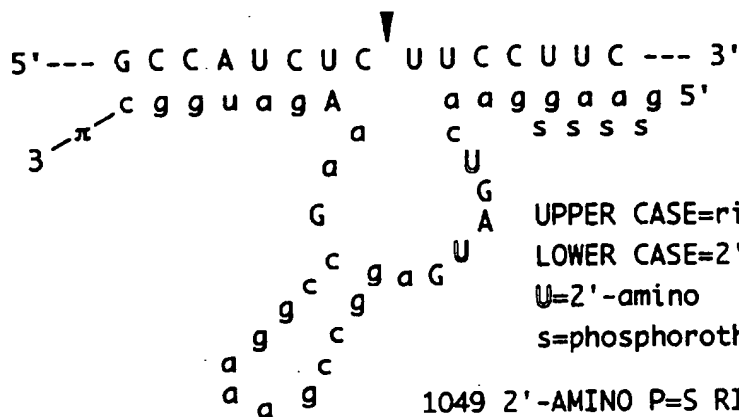


FIG. 18a.

UPPER CASE=ribonucleotides  
 LOWER CASE=2'-O-Methylnucleotides  
 U=2'-amino  
 s=phosphorothioatelinkages

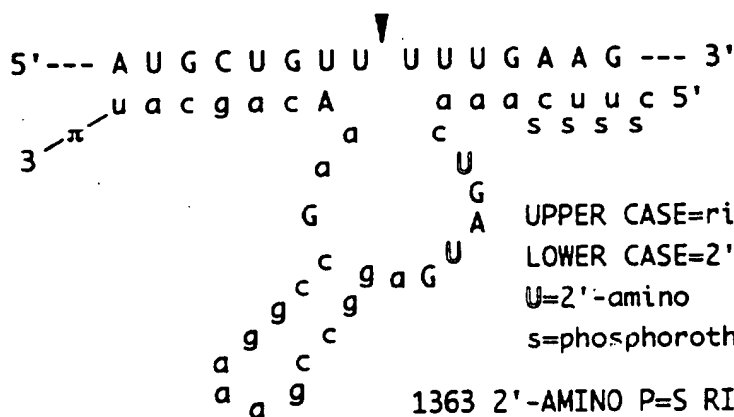


FIG. 18b.

UPPER CASE=ribonucleotides  
 LOWER CASE=2'-O-Methylnucleotides  
 U=2'-amino  
 s=phosphorothioatelinkages

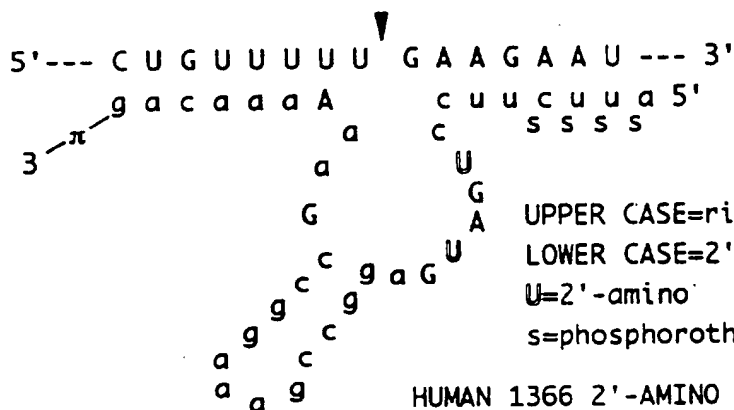


FIG. 18c.

UPPER CASE=ribonucleotides  
 LOWER CASE=2'-O-Methylnucleotides  
 U=2'-amino  
 s=phosphorothioatelinkages

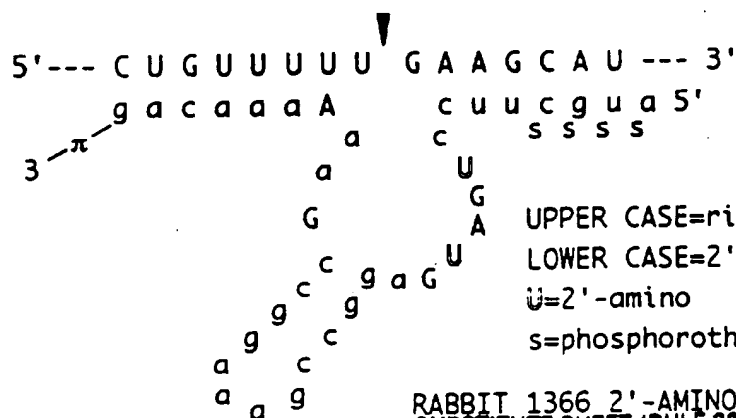


FIG. 18d.

UPPER CASE=ribonucleotides  
 LOWER CASE=2'-O-Methylnucleotides  
 U=2'-amino  
 s=phosphorothioatelinkages

FIG. 19.

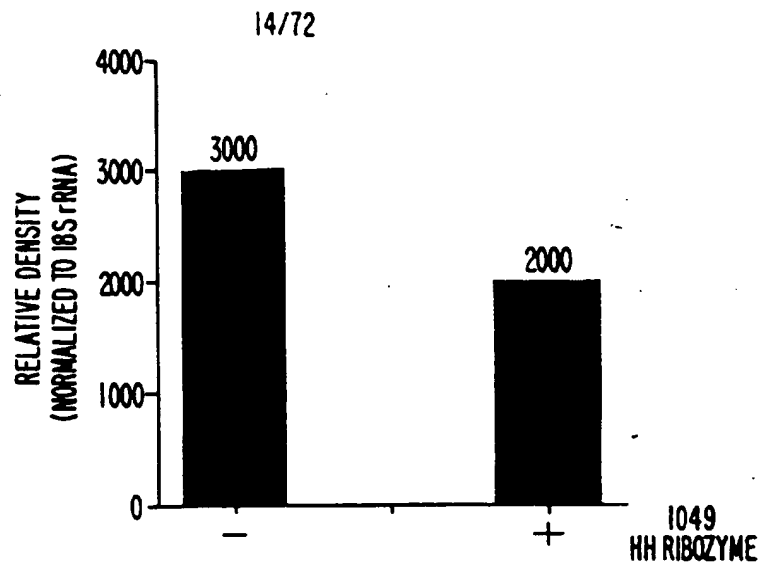


FIG. 20.

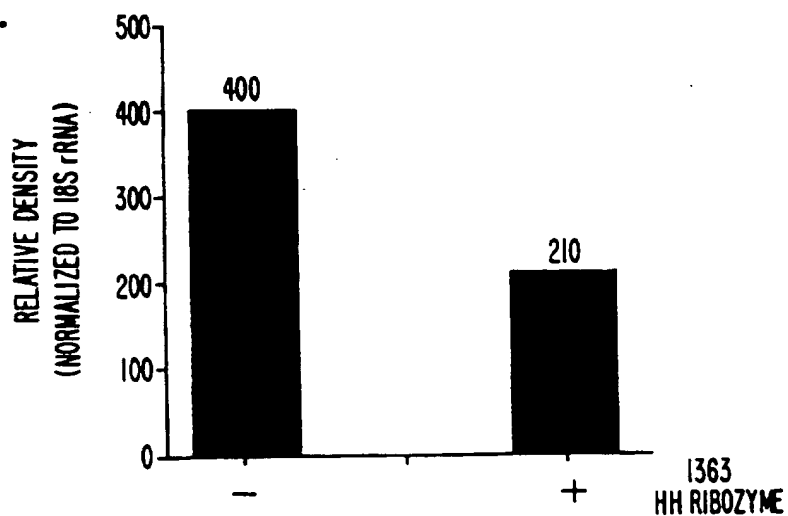
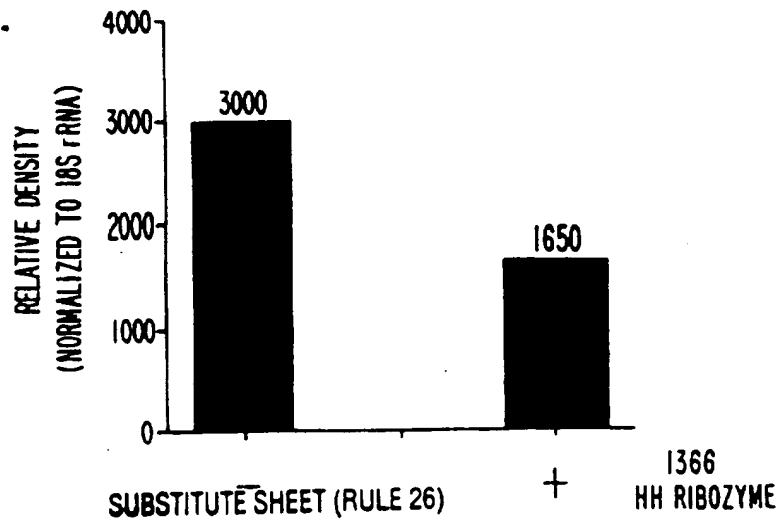


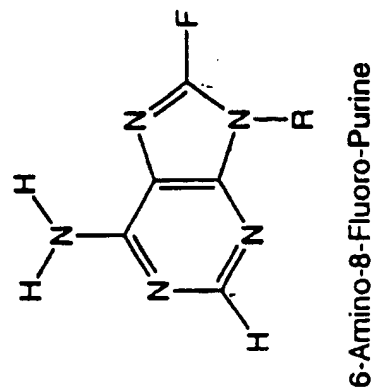
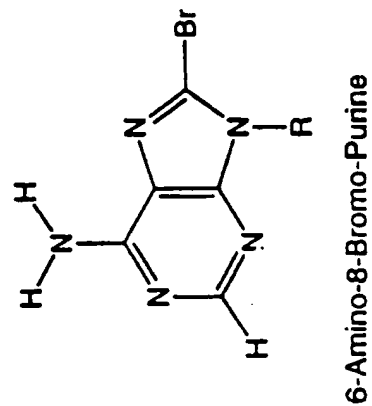
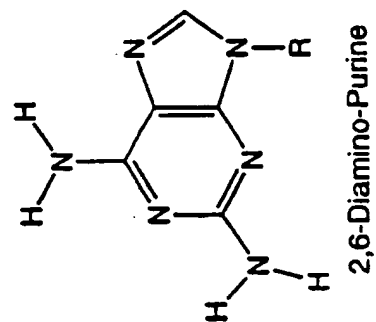
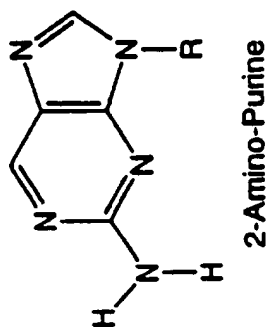
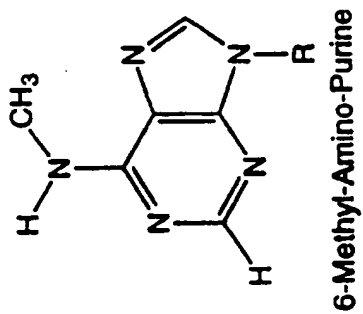
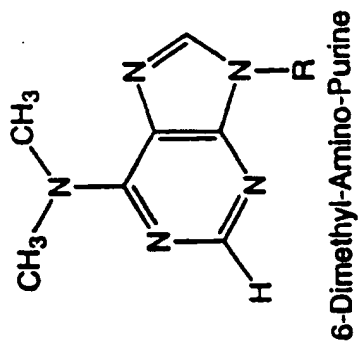
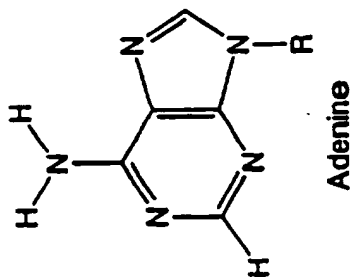
FIG. 21.





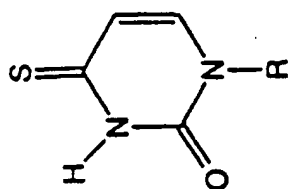
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FIG. 22a.

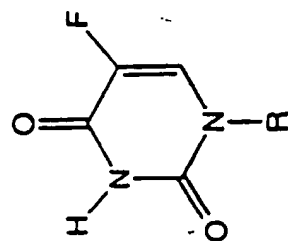


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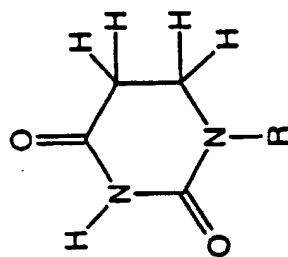
FIG. 22b.



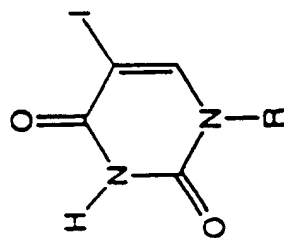
4-Thio-Uracil



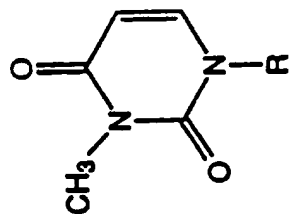
5-Fluoro-Uracil



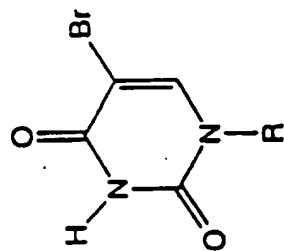
5,6-Dihydro-Uracil



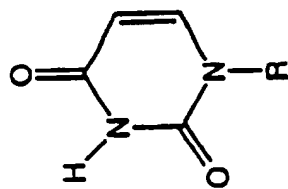
5-Iodo-Uracil



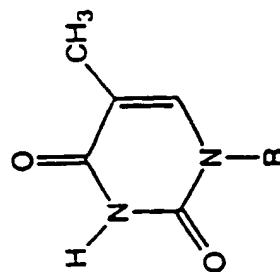
3-Methyl-Uracil



5-Bromo-Uracil



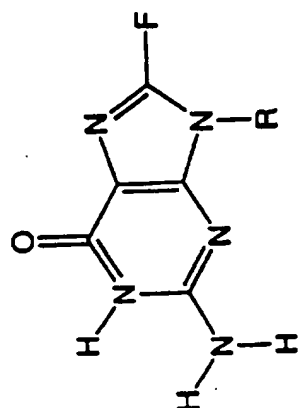
Uracil



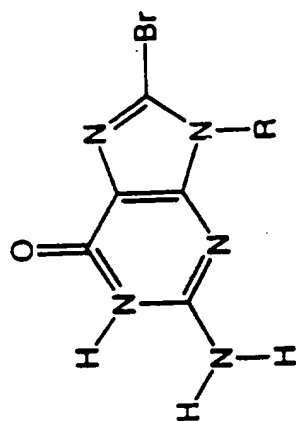
Thymine

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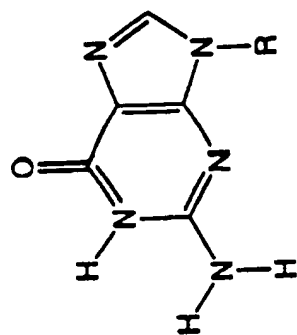
FIG. 22c.



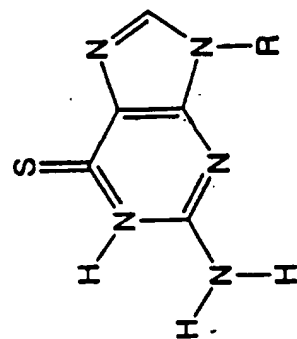
8-Fluoro-Guanine



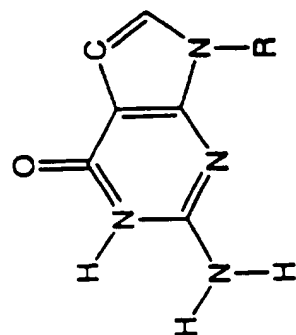
8-Bromo-Guanine



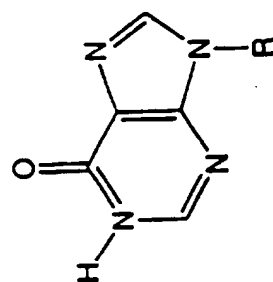
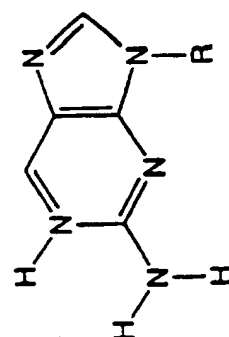
Guanine



6-Thio-Guanine



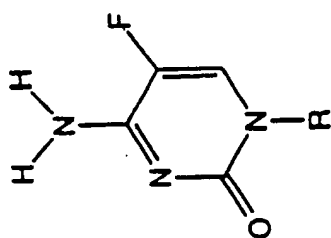
7-Deaza-Guanine

Hypoxanthine  
(Inosine base)

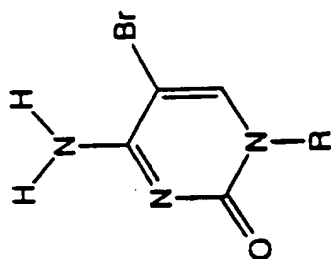
2-Amino-Purine

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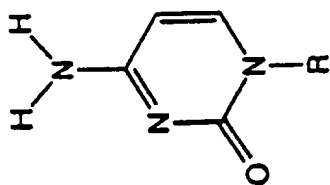
FIG. 22d.



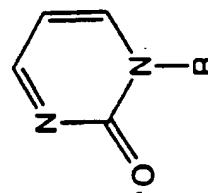
5-Fluoro-Cytosine



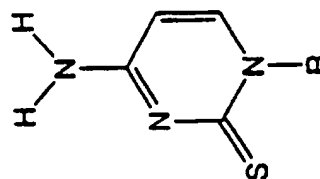
5-Bromo-Cytosine



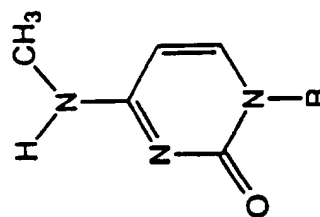
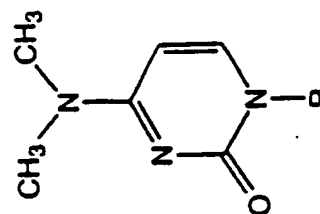
Cytosine



2-Pyridone



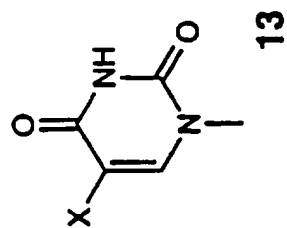
2-Thio-Cytosine

N<sup>4</sup>-Methyl-CytosineN<sup>4</sup>,N<sup>4</sup>-dimethyl-Cytosine

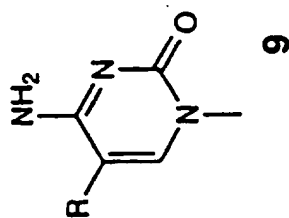


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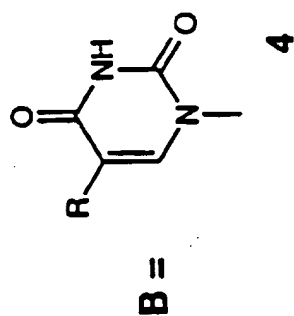
FIG. 24.



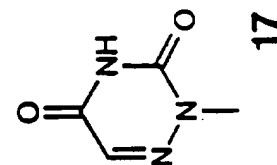
5-Halouridine



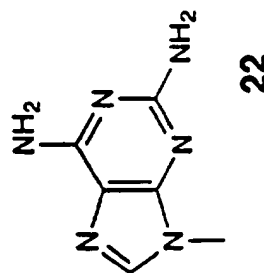
5-Alkylcytidine



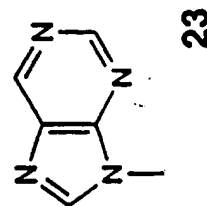
5-Alkyluridine



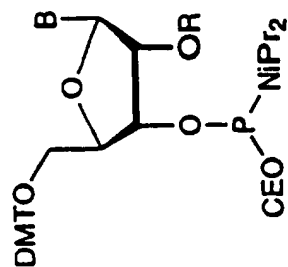
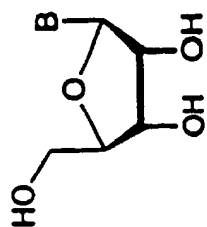
6-Azauridine



Diaminopurine

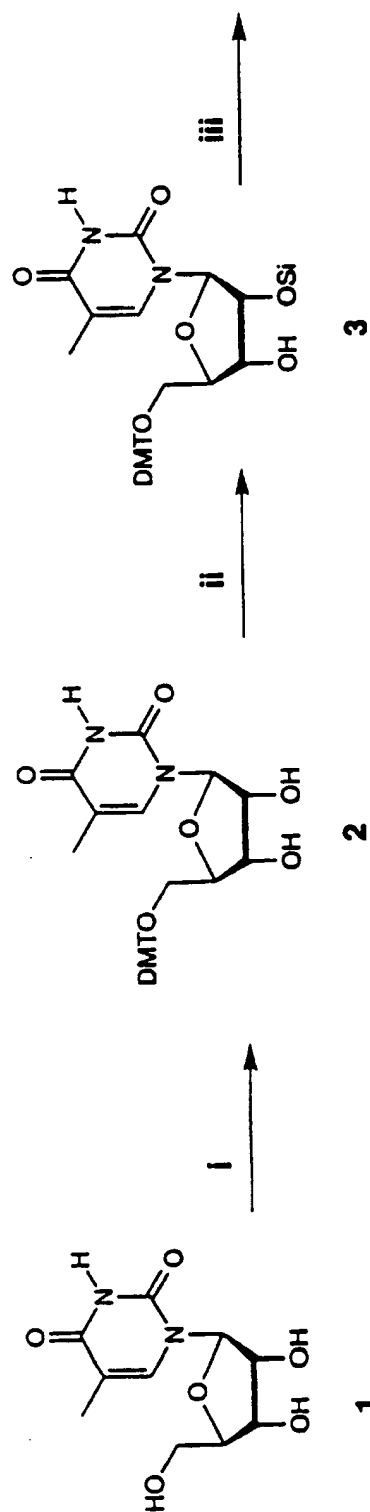


Purine

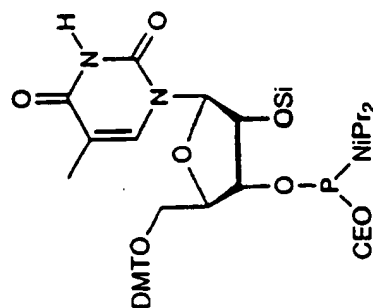


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FIG. 25.



i) = DMT-Cl/Pyr  
 ii) = TBDMS-Cl/Pyr,  $\text{Bu}_4\text{NNO}_3$   
 iii) = P(OCE)( $\text{NiPr}_2$ )/THF, sym-collidine



4

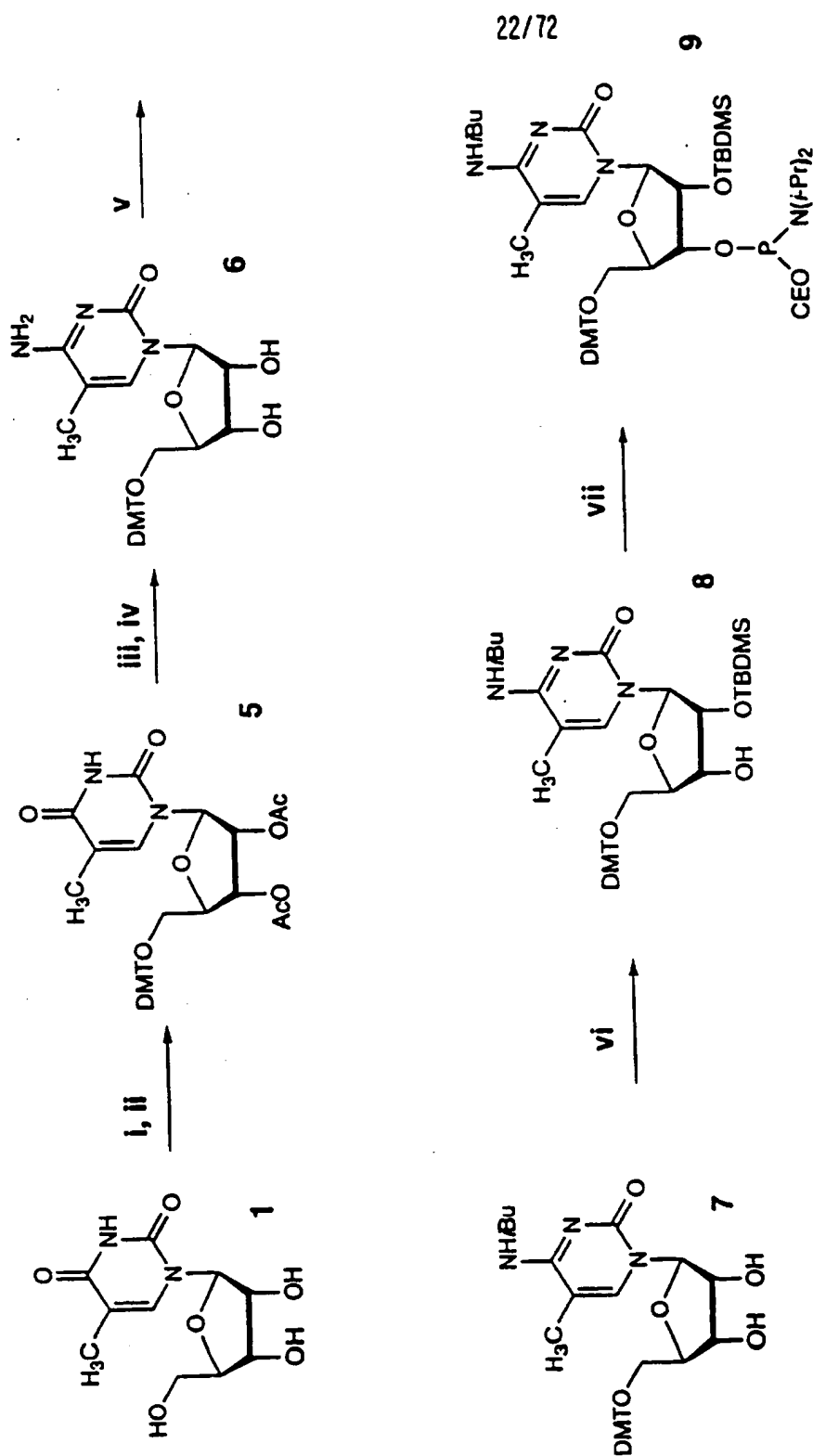
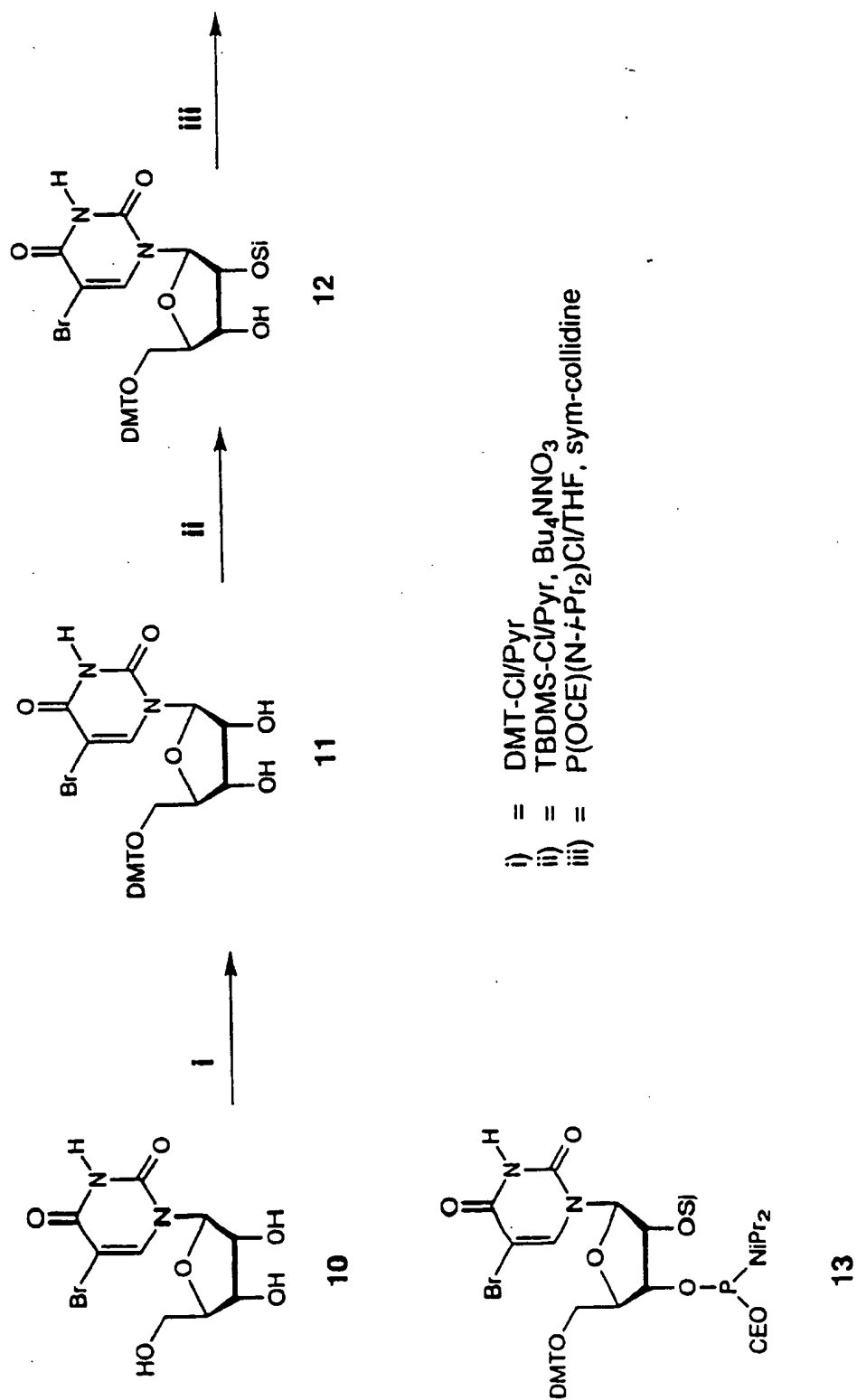


FIG. 26.



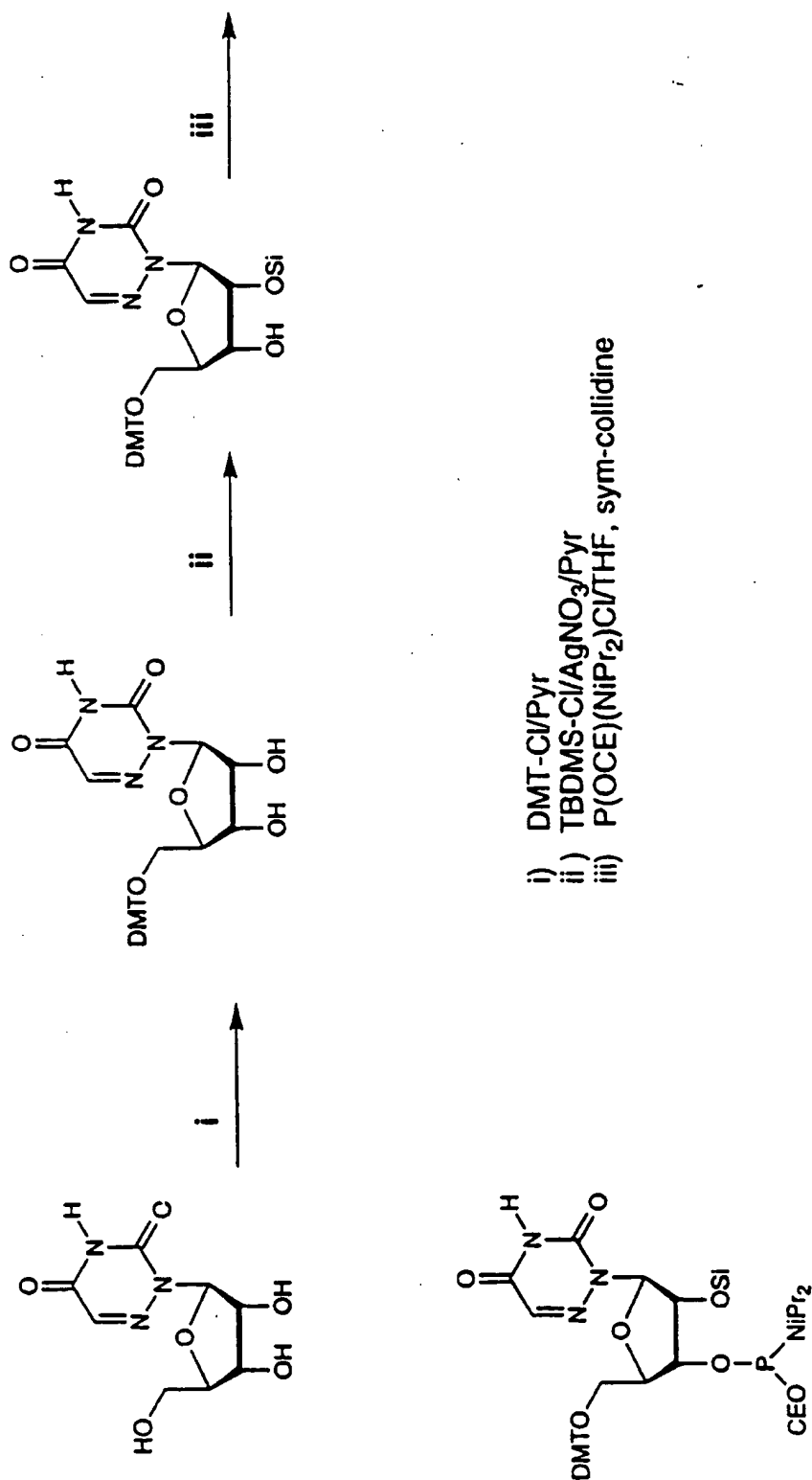
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FIG. 27.

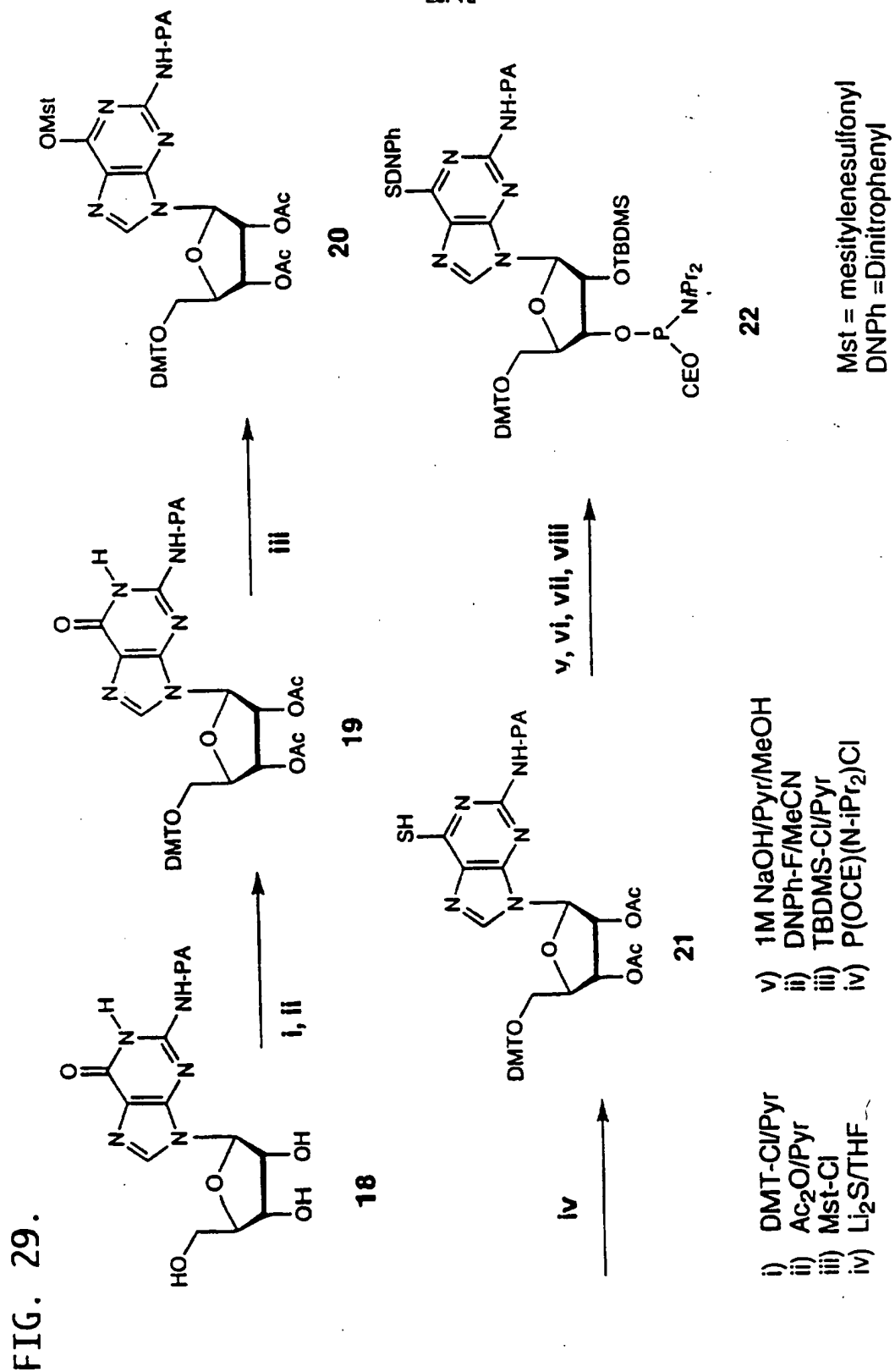


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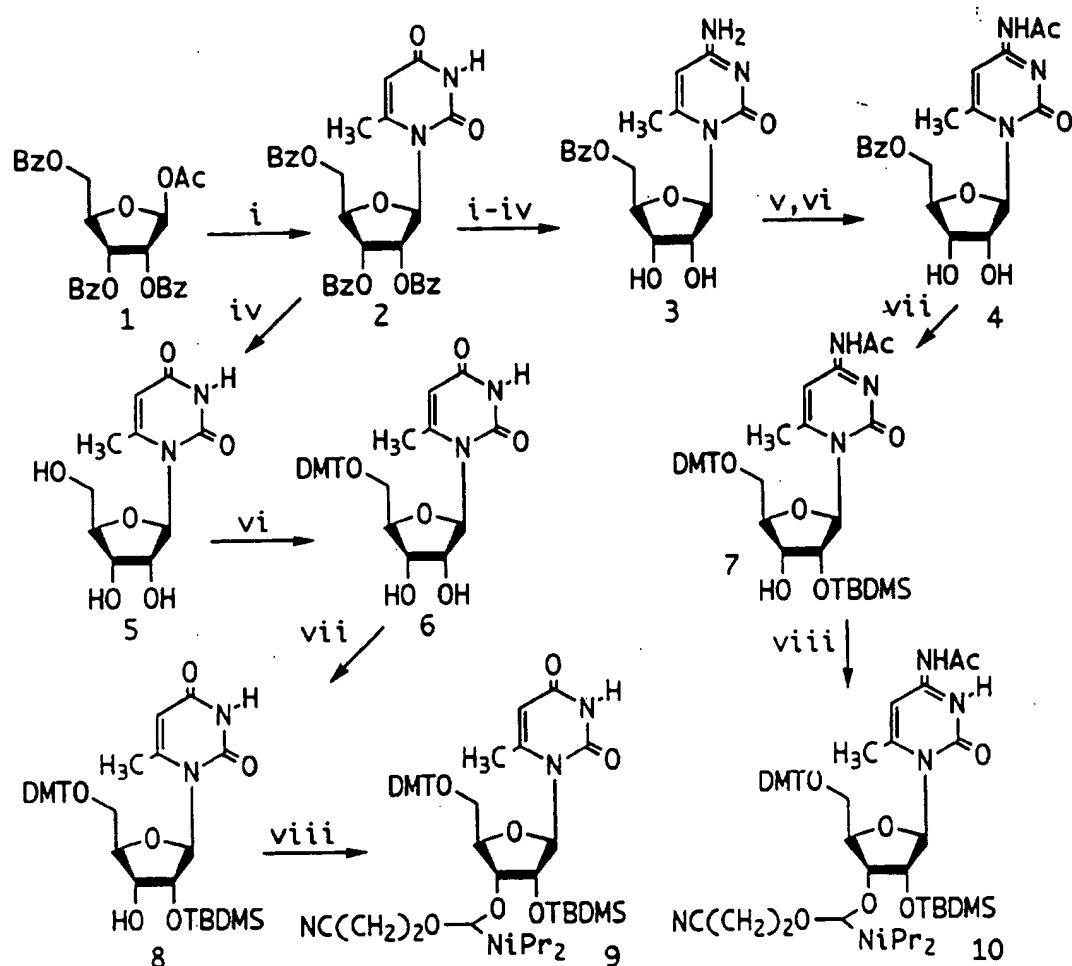
FIG. 28.



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## REAGENTS AND CONDITIONS:

- i) 6-Me-Ura<sup>TMS</sup>, CF<sub>3</sub>SO<sub>3</sub>SIME<sub>3</sub>, 0°C;
- ii) 1,2,4-triazole, POCl<sub>3</sub>; iii) NH<sub>4</sub>OH/dioxane;
- iv) 2M NaOH/Pyr/MeOH; v) MeSI-CL/PYR, THEN AC<sub>2</sub>O;
- vi) DMT-Cl/Pyr;
- vii) TBDMS-Cl/AgNO<sub>3</sub>/Pyr/THF;
- viii) 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite, DIPEA/CH<sub>2</sub>Cl<sub>2</sub>.

FIG. 30.

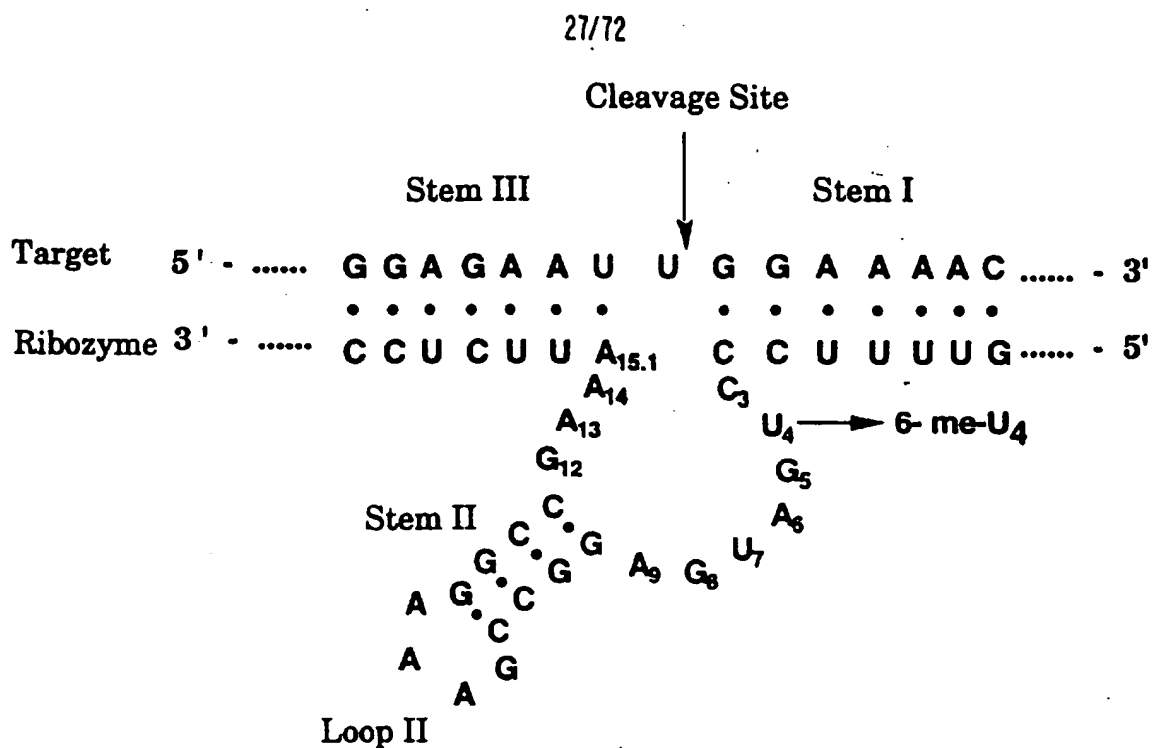
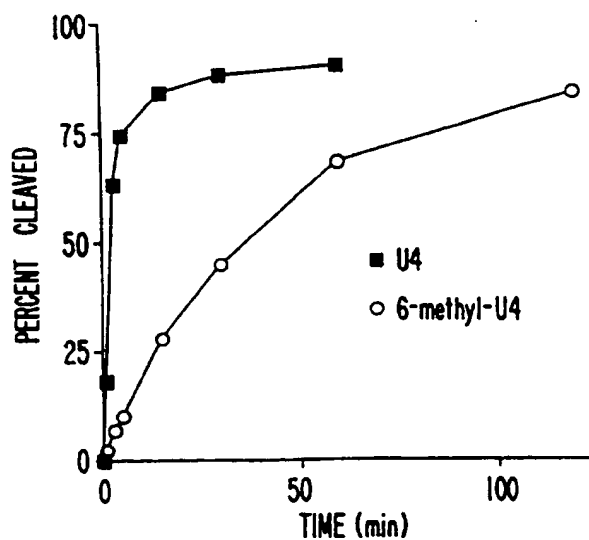


FIG. 31.



[Ribozyme]=40nM [Substrate]=~1nM

FIG. 32.

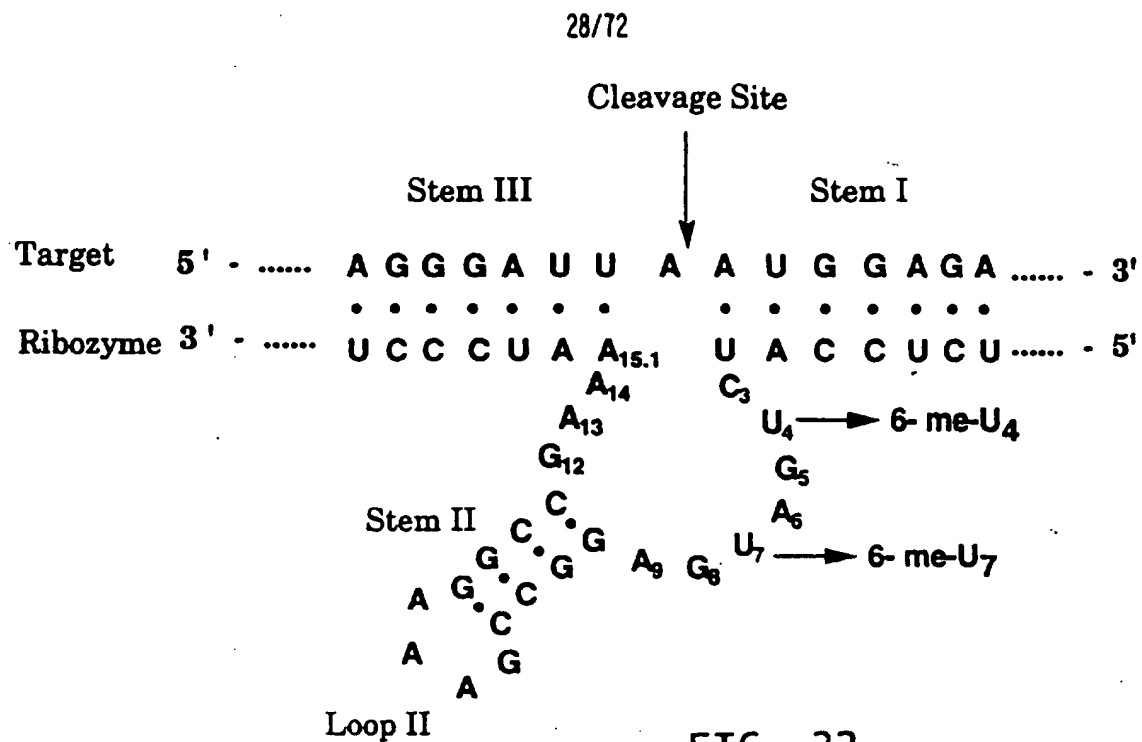
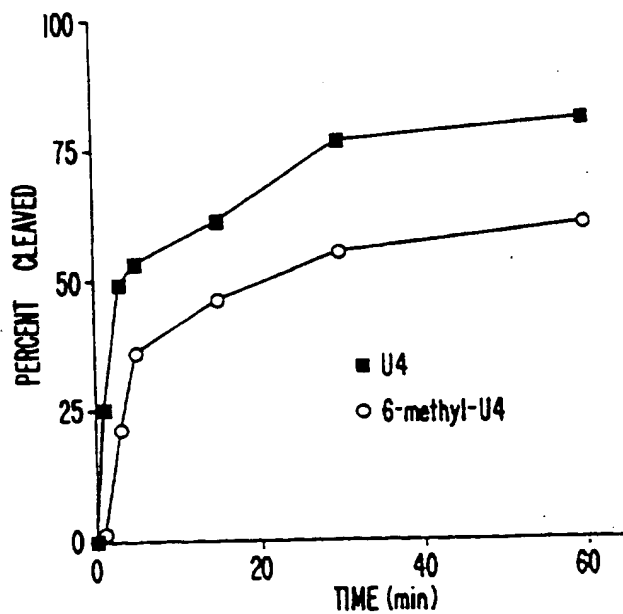


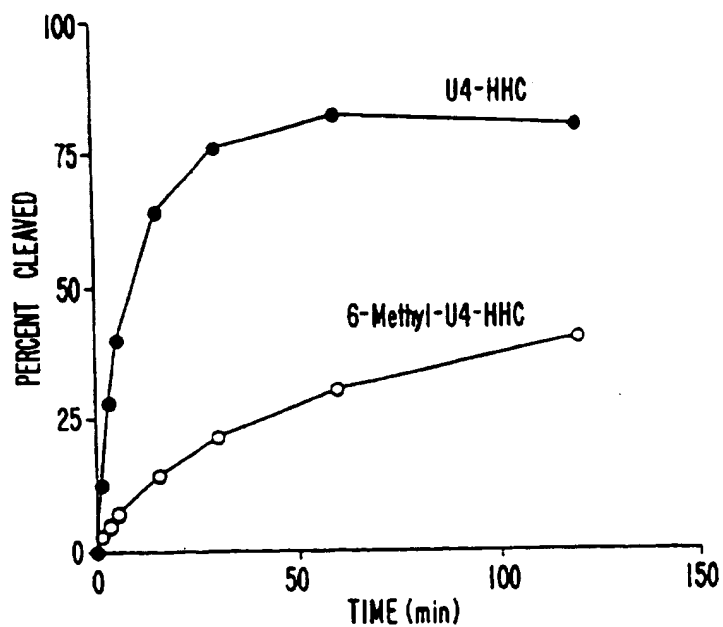
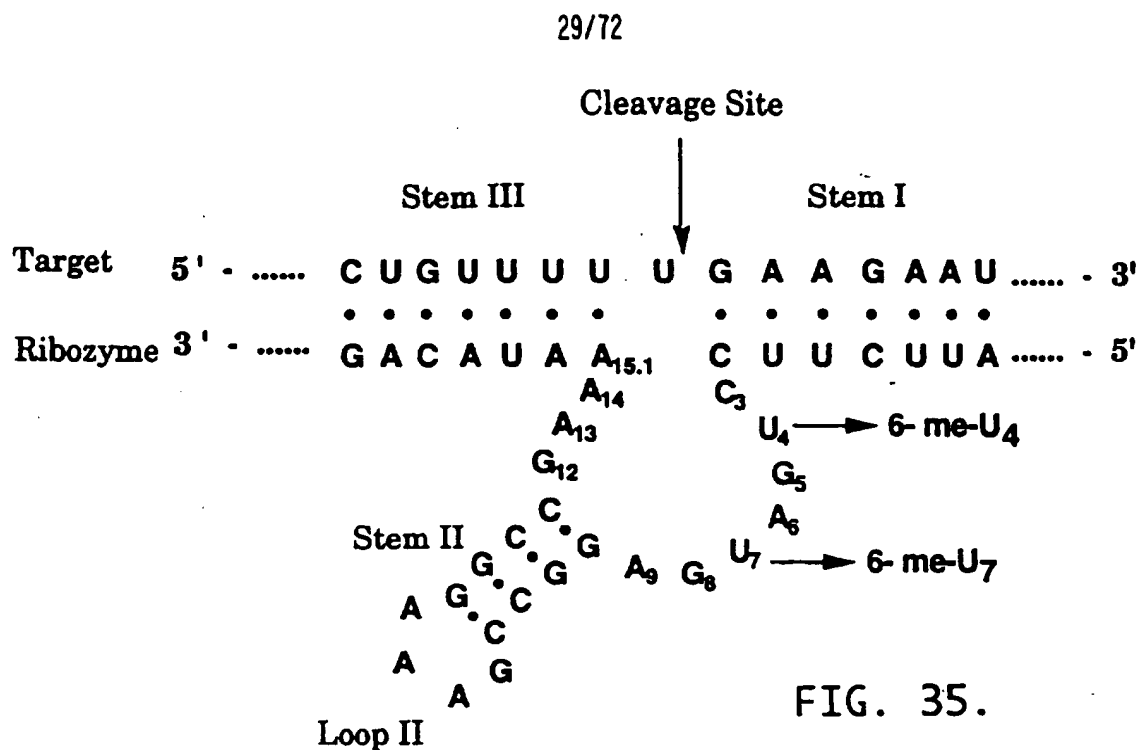
FIG. 33.



[Ribozyme]=40nM [Substrate]=~1nM

FIG. 34.

SUBSTITUTE SHEET (RULE 26)



[Ribozyme]=40nM [Substrate]=~1nM

FIG. 36.  
SUBSTITUTE SHEET (RULE 26)

FIG. 37.

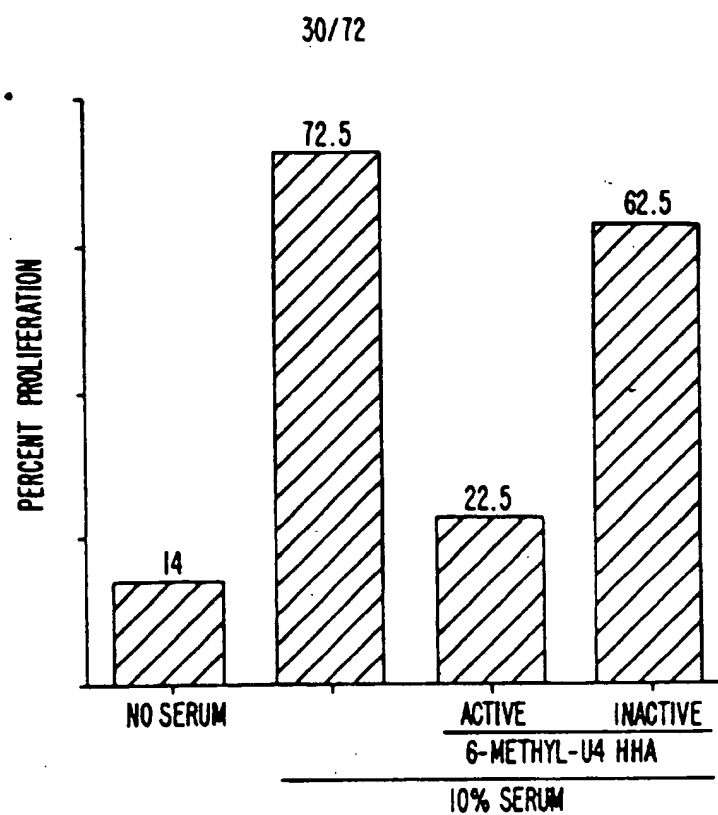
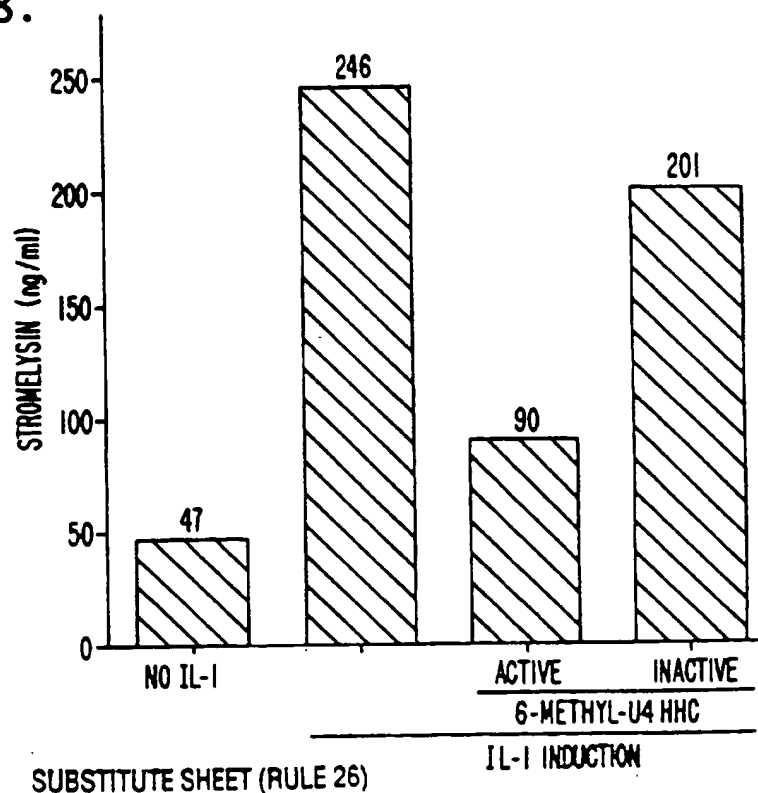
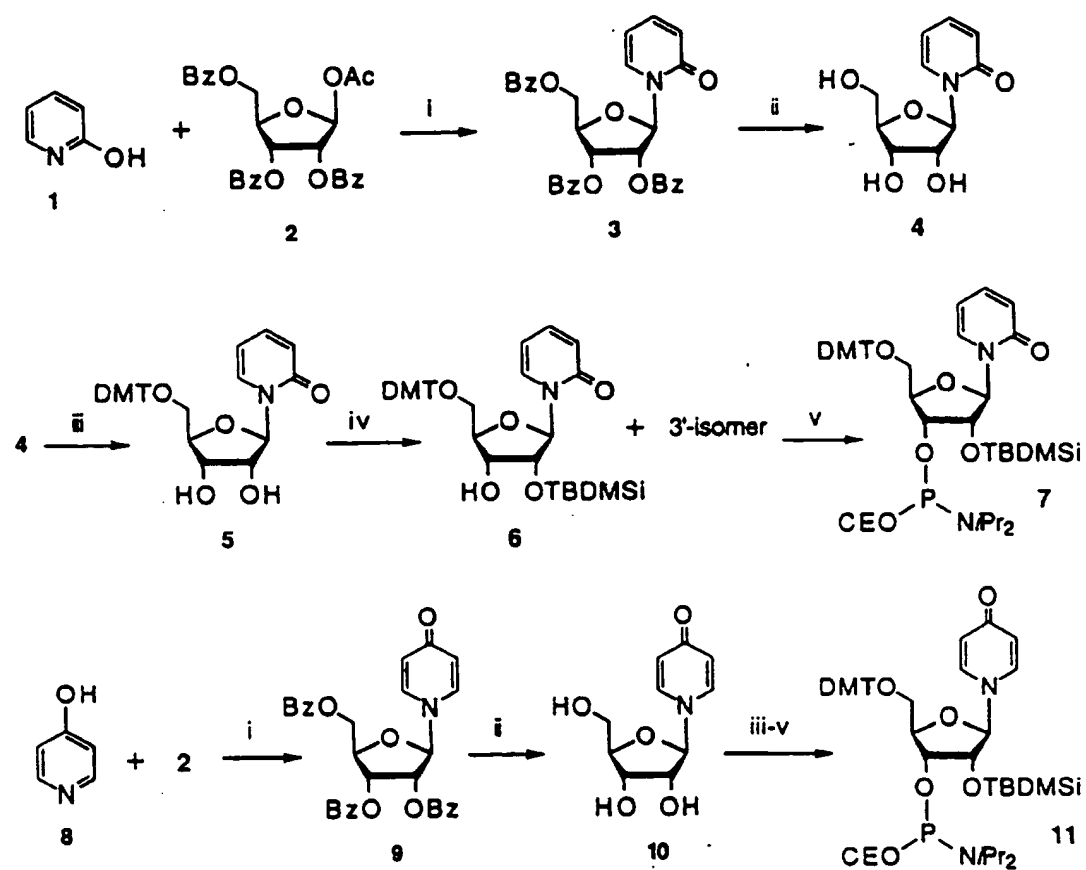


FIG. 38.





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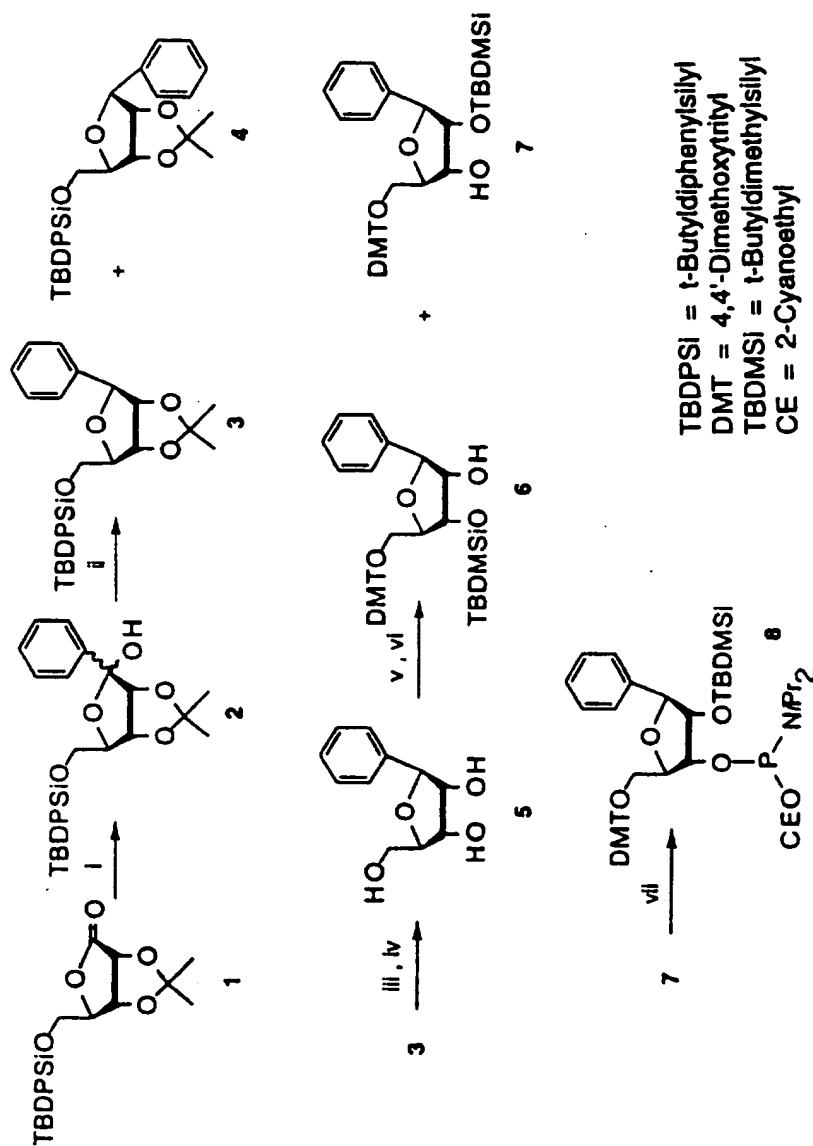


Bz = Benzoyl  
 Ac = Acetyl  
 DMT = 4,4'-Dimethoxytrityl  
 TBDMSi = *t*-Butyldimethylsilyl  
 CE = 2-cyanoethyl

Reagents and Conditions: i: N,O-bis(trimethylsilyl)acetamide (BSA)/TMSTf/  
 CH<sub>3</sub>CN, 70 °C, ii: NaOCH<sub>3</sub>/CH<sub>3</sub>OH, iii: DMT-Cl/DMAP/Et<sub>3</sub>N/Pyr, iv: TBDMSi-  
 Cl/AgNO<sub>3</sub>/Pyr/THF, v: P(OCE)(N-*i*Pr<sub>2</sub>)Cl/DIPEA/1-MeIm/CH<sub>2</sub>Cl<sub>2</sub>.

FIG. 39.

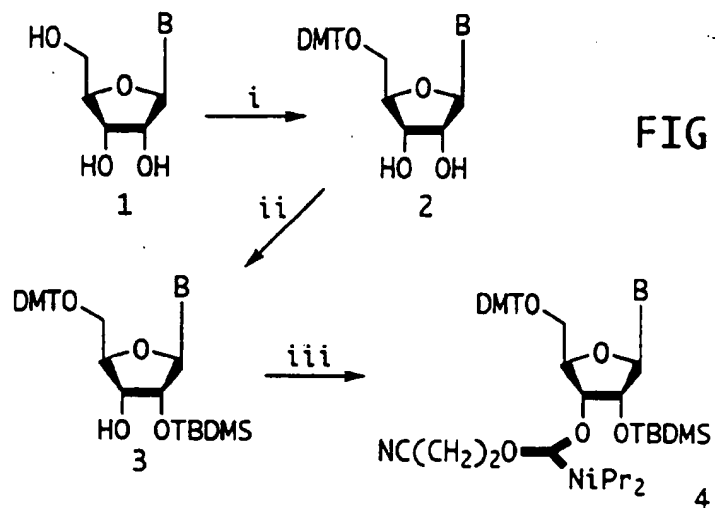
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Reagents and Conditions: i:  $\text{PhLi/THF}$ ,  $-78^\circ\text{C}$ ; ii:  $\text{Et}_3\text{SiH/BF}_3\cdot\text{Et}_2\text{O/CH}_3\text{CN}$ ,  $-40^\circ\text{C}$ ; iii:  $1\text{M TBAF/THF}$ ; iv:  $70\% \text{ aq. CH}_3\text{COOH}$ ; v:  $\text{DMT-Cl/DMAP/Et}_3\text{N/Pyr}$ ; vi:  $\text{TBDMSi-Cl/AgNO}_3/\text{Pyr/THF}$ ; vii:  $\text{P(OCE)(N-iPr}_2\text{)Cl/DIPEA/1-MeIm/CH}_2\text{Cl}_2$ .

FIG. 40.

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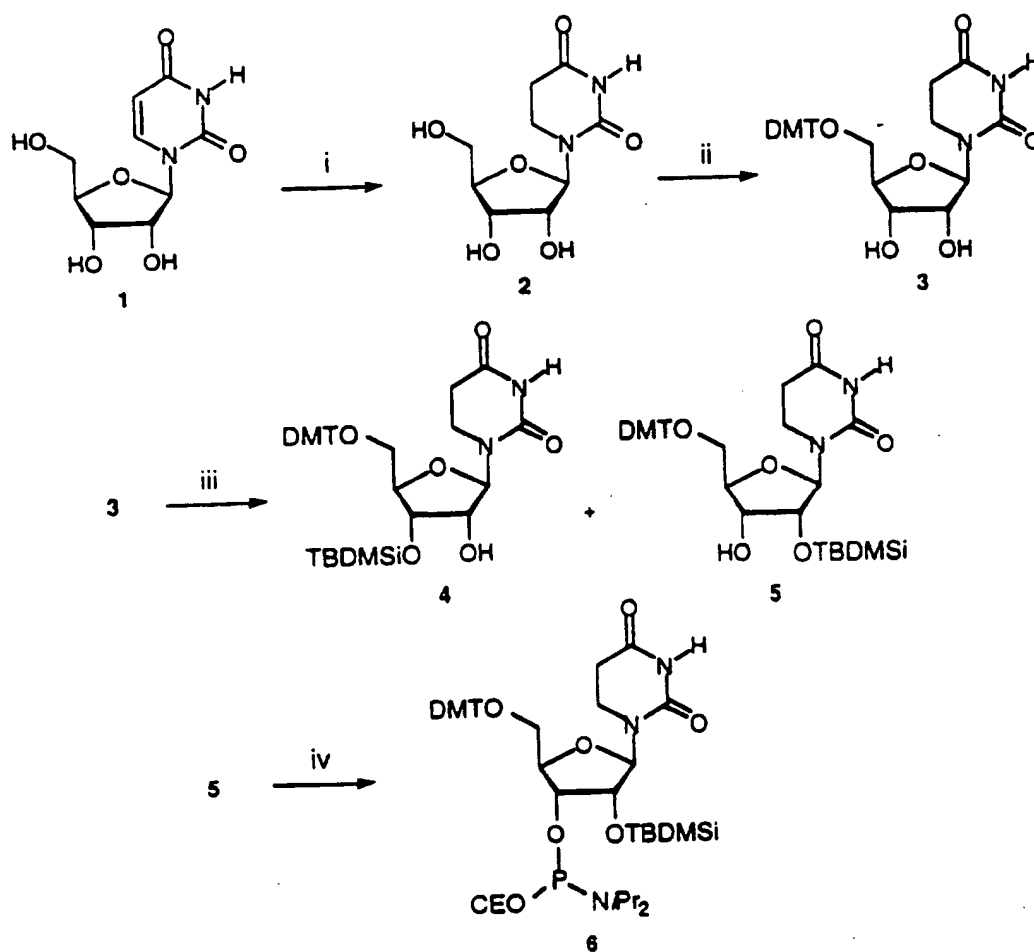
B=Pseudo U,2,4,6-trimethoxy benzene or 3-methyl U

REAGENTS AND CONDITIONS:

- i) DMT-Cl/Pyr;
- ii) TBDMS-Cl/AgNO<sub>3</sub>/Pyr/THF;
- iii) 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite, DIPEA/CH<sub>2</sub>Cl<sub>2</sub>.

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FIG. 42.



DMT = 4,4'-Dimethoxytrityl  
 TBDMSi = t-Butyldimethylsilyl  
 CE = 2-Cyanoethyl

**Reagents and Conditions:** i: Pd/Rh, H<sub>2</sub> 60 psi, ii: DMT-Cl/DMAP/Et<sub>3</sub>N/Pyr,  
 iii: TBDMSi-Cl/AgNO<sub>3</sub>/Pyr/THF, iv: P(OCE)(N-iPr<sub>2</sub>)/Cl/DIPEA/1-Melm/CH<sub>2</sub>Cl<sub>2</sub>

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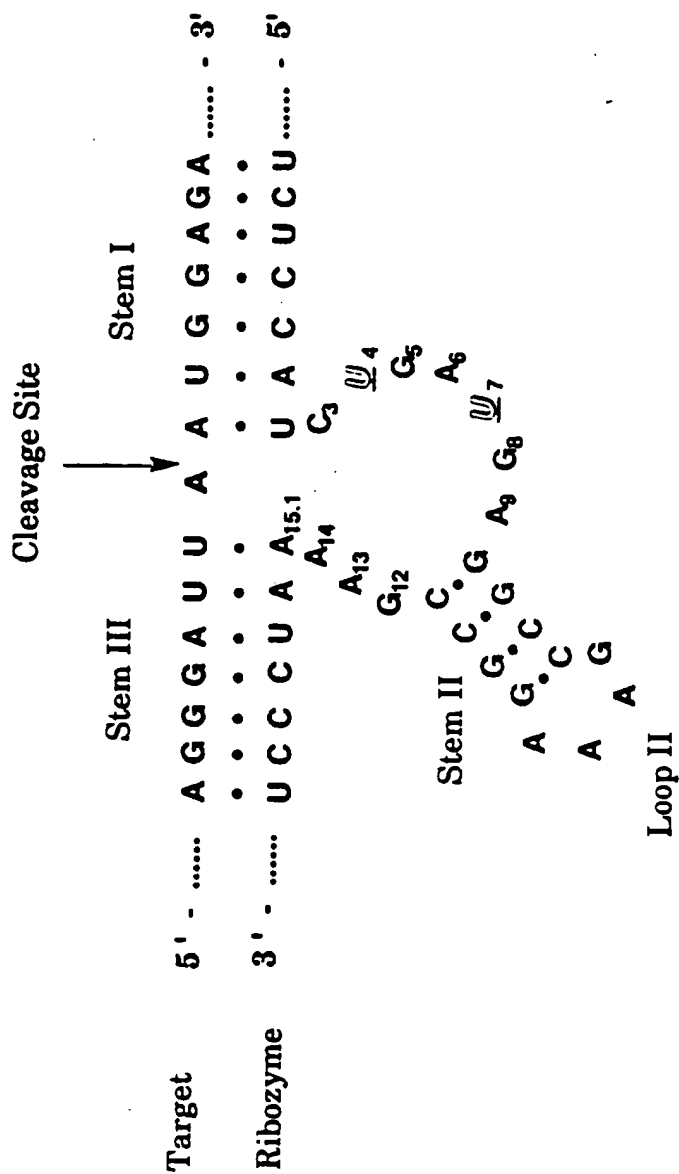
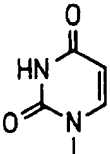
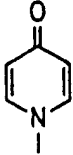
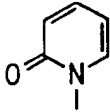

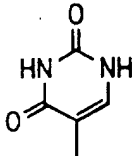
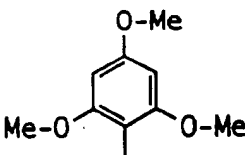
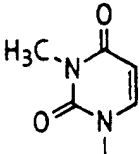


FIG. 43a.

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FIG. 43b.

	BASE MODIFICATIONS	HH nt. POSITION	
		<u>4</u>	<u>7</u>
		<u>k<sub>obs</sub>(min<sup>-1</sup>)</u>	
	U	2.1	2.1
	Pyridin-4-one	0.04	≥10
	Pyridin-2-one	0.03	1.2
	Phenyl	0.05	2.5
	Pseudo U	1.0	0.22
	3-O-Methoxy Benzene	0.02	0.14
	3-Me thyl U	0.02	4.6

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FIG. 44a.

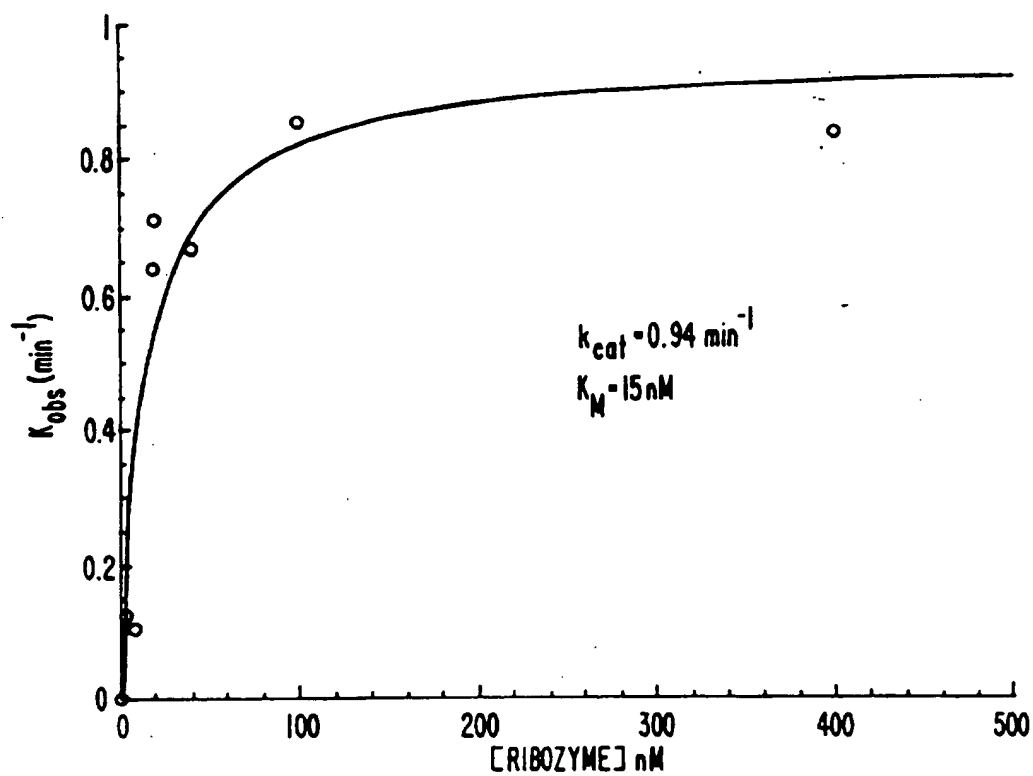
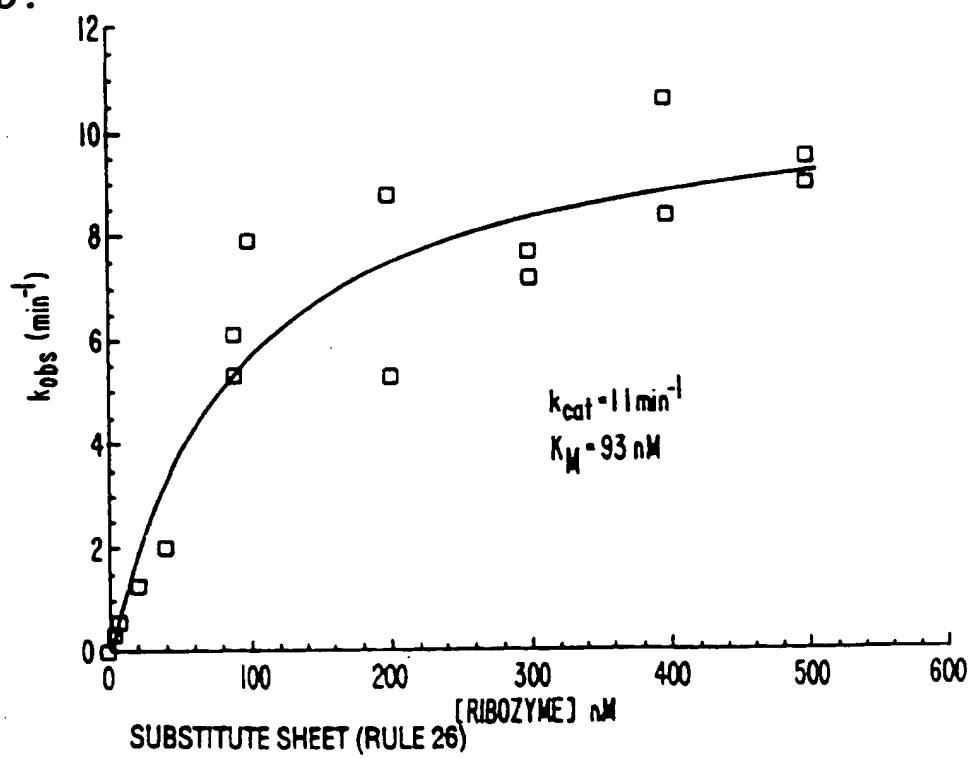


FIG. 44b.



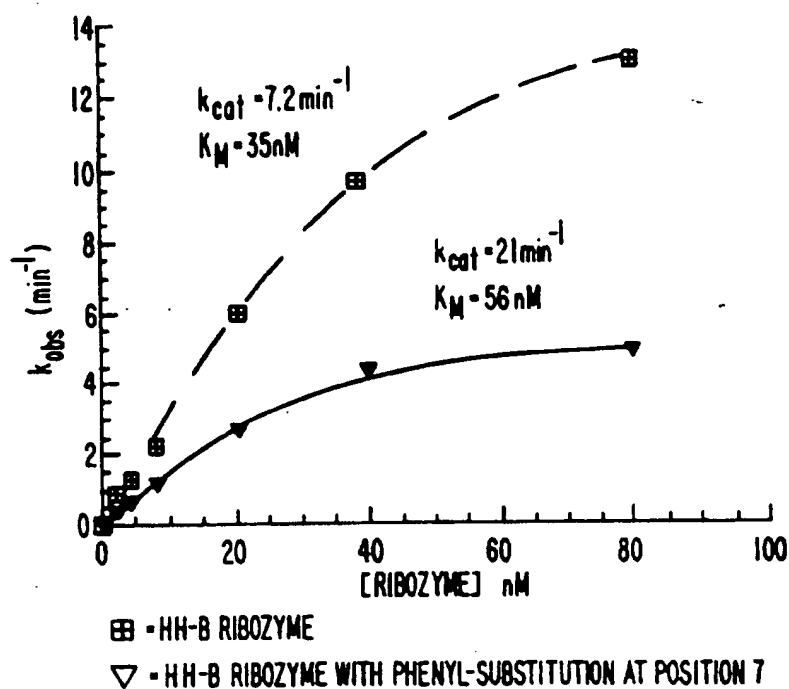
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FIG. 44c.

FIG. 45.

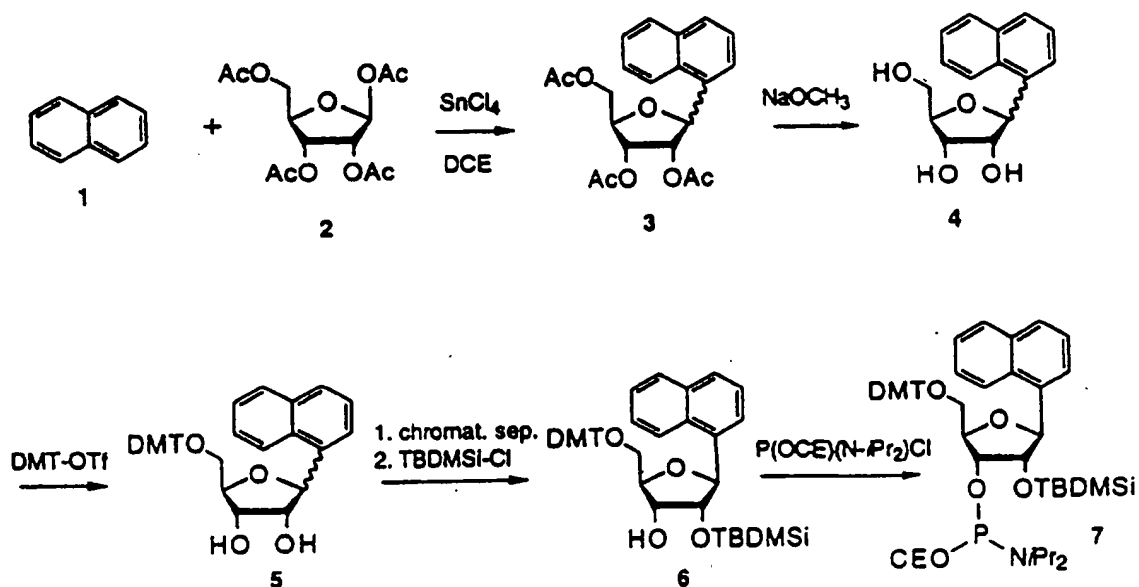
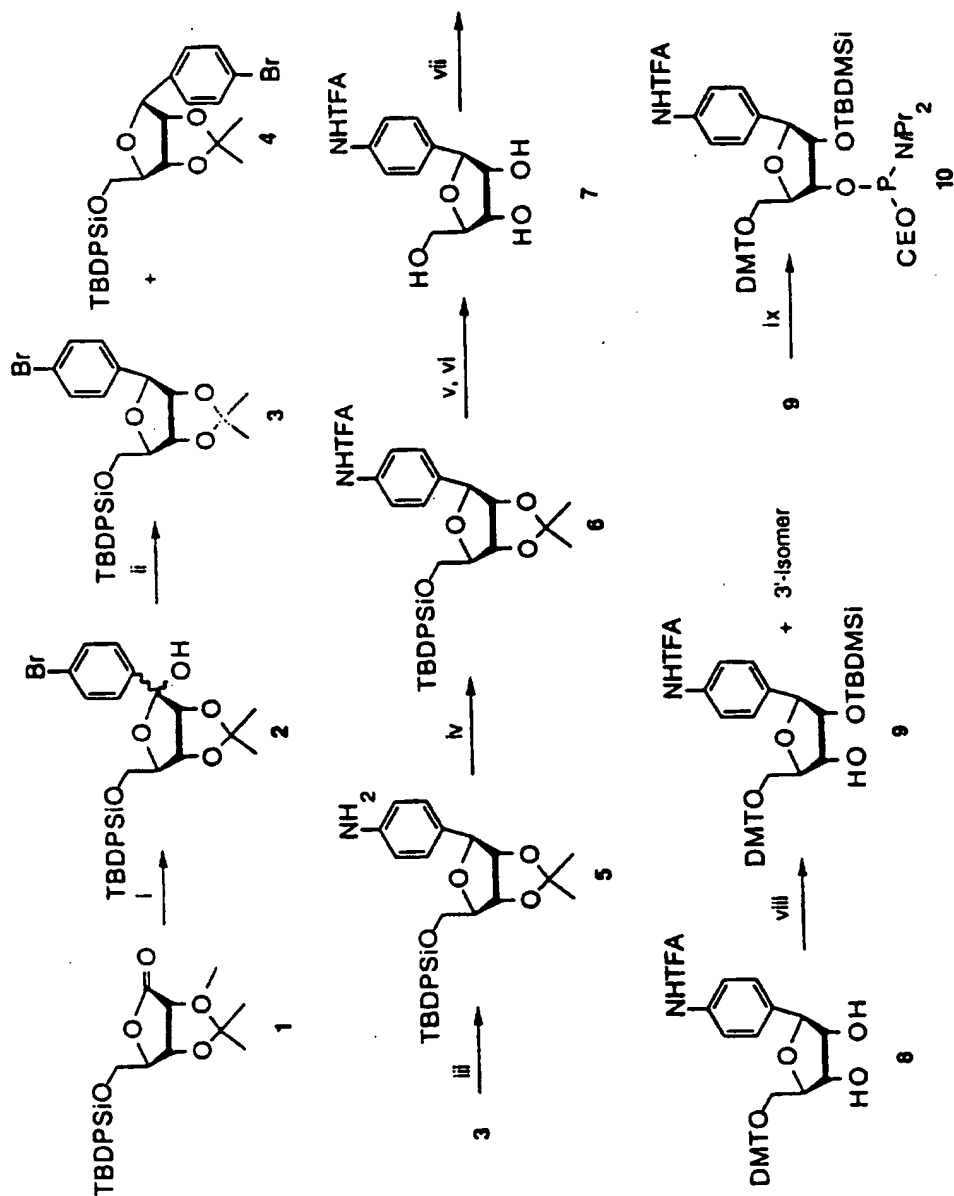




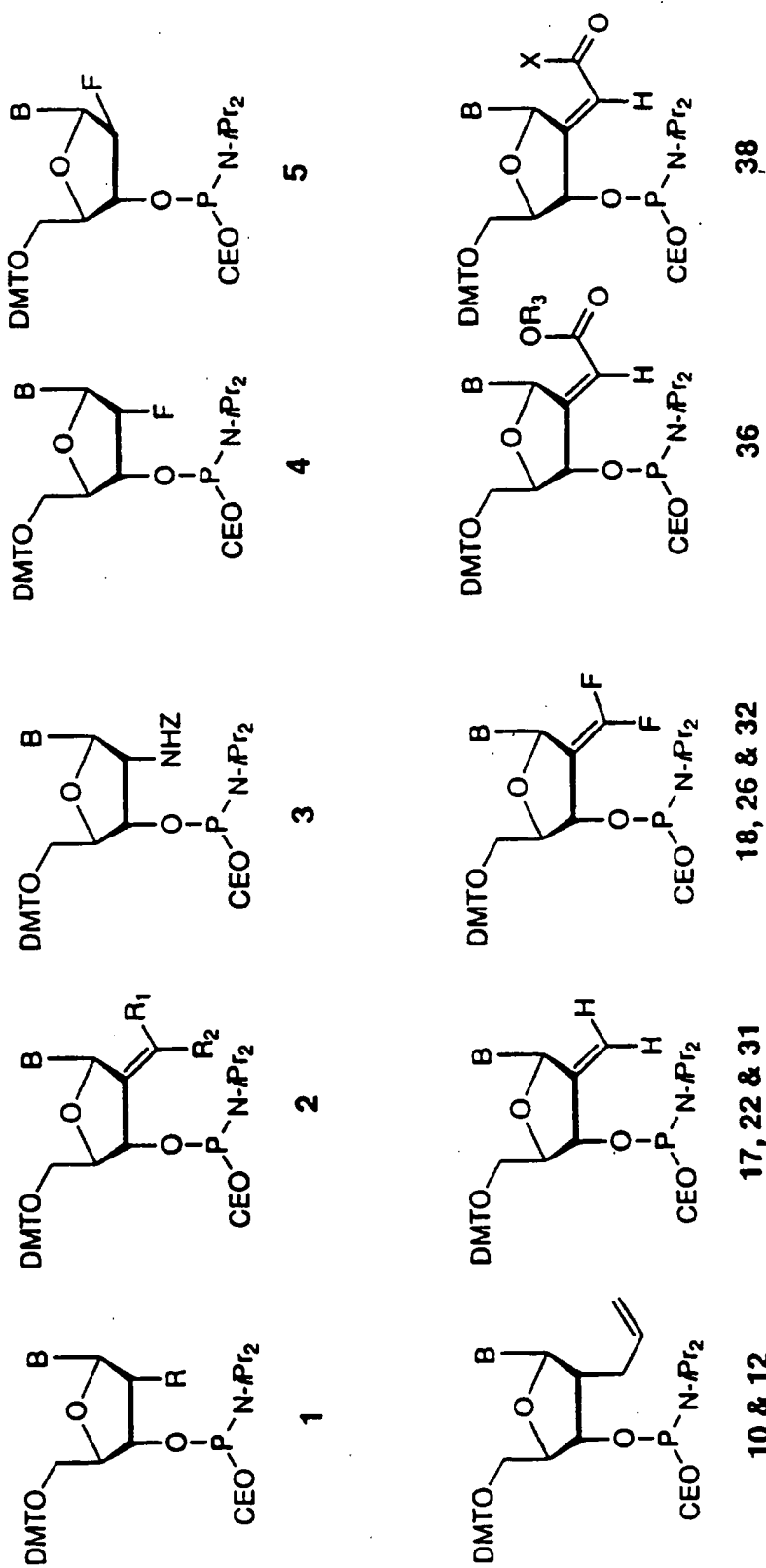
FIG. 46.



Reagents and Conditions: i: 1-Li-4-bromobenzene/THF, -78 °C, ii: Et<sub>3</sub>SiH/BF<sub>3</sub>·Et<sub>2</sub>O/CH<sub>3</sub>CN, -40 °C, iii: liq. NH<sub>3</sub>/CuI, 115 °C, iv: TFA<sub>2</sub>O/Pyr, v: 1M TBAF/THF, vi: 70% aq. CH<sub>3</sub>COOH, 100 °C, vii: DMT-CI/DMAPI/Et<sub>3</sub>N/Pyr, viii: TBDMSi-CI/AgNO<sub>3</sub>/Pyr/THF, ix: P(OCE)(N-iPr<sub>2</sub>)CI/DIPEA/1-Melm/CH<sub>2</sub>Cl<sub>2</sub>.



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B = Protected A, C, G, U, T, 2AP, I, DiAP, P etc.

FIG. 48.

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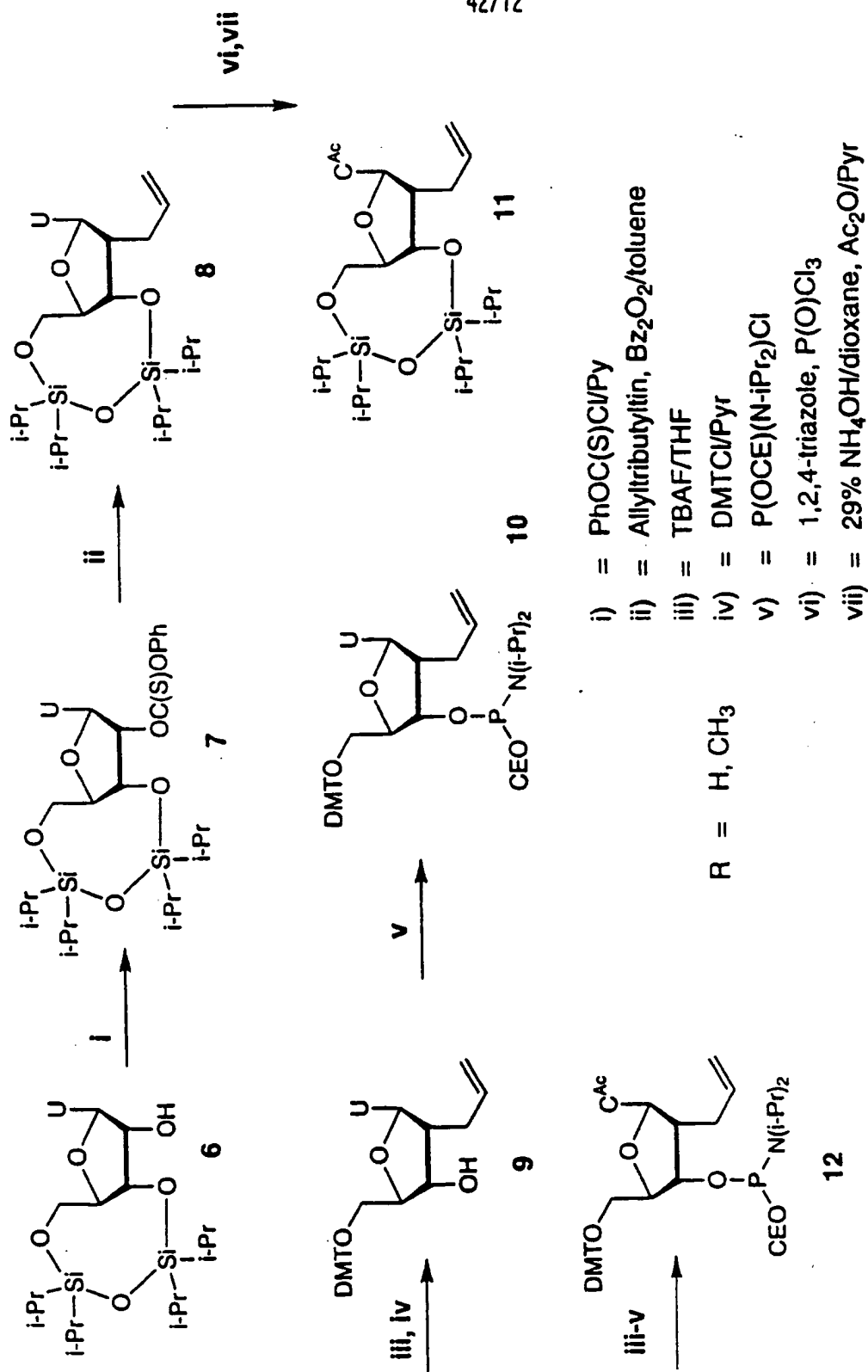


FIG. 49.

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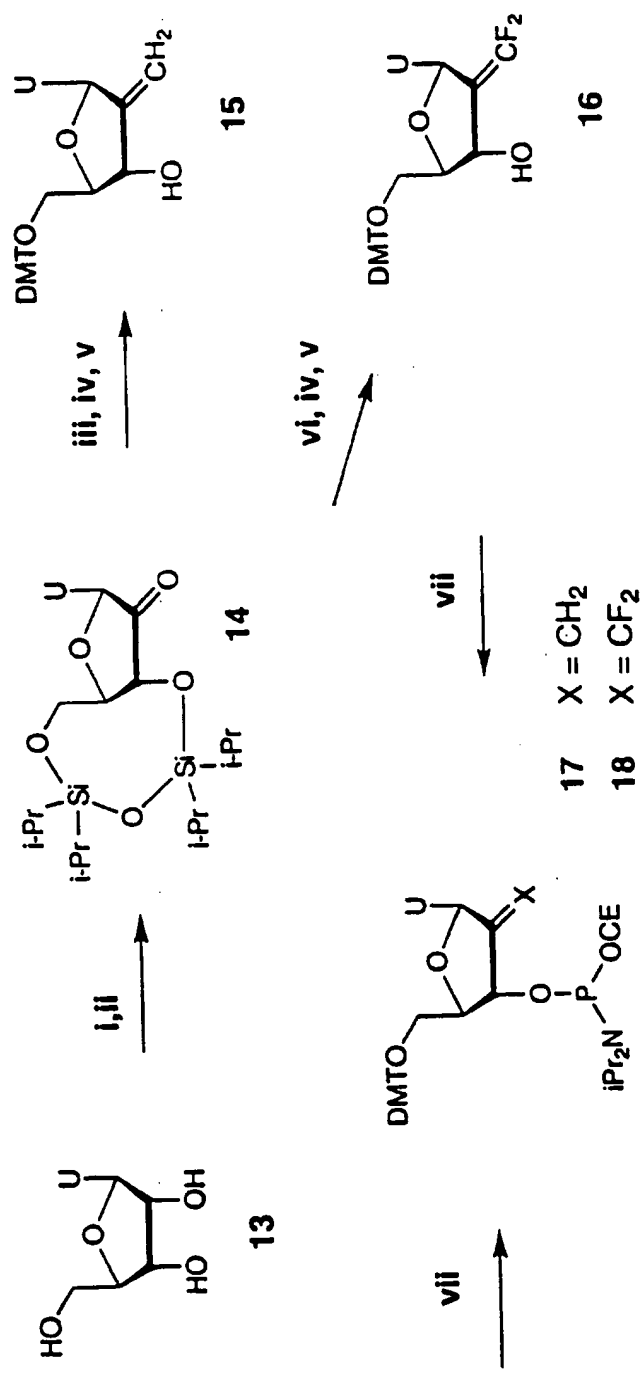


FIG. 50.

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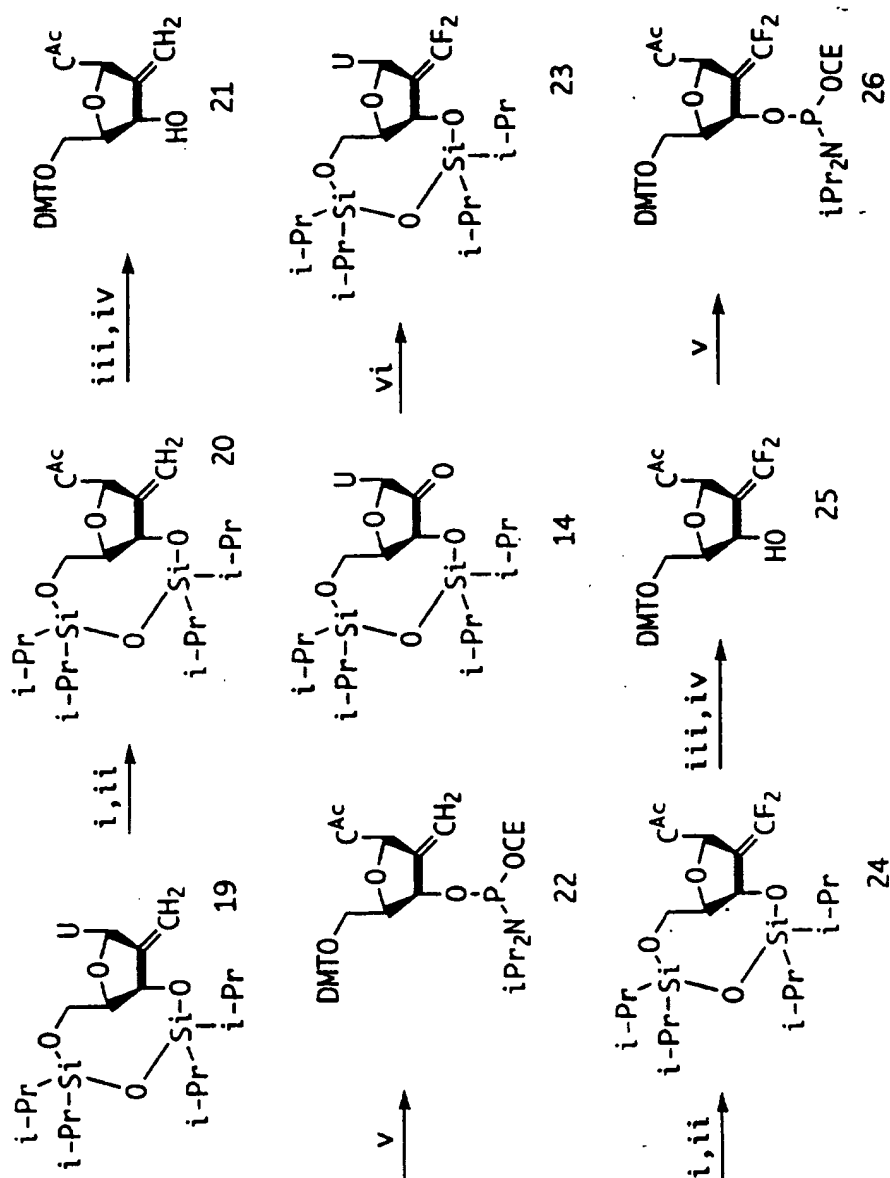


FIG. 51.

i) = 1,2,4-triazole, P(O)Cl<sub>3</sub>      iii) = TBAF/THF      v) = P(OCE)(N-iPr)<sub>2</sub>Cl  
 ii) = 29% NH<sub>4</sub>OH/dioxane, Ac<sub>2</sub>O/Pyr      iv) = DMTCl/Pyr      vi) = Ph<sub>3</sub>P, ClCF<sub>2</sub>COONa

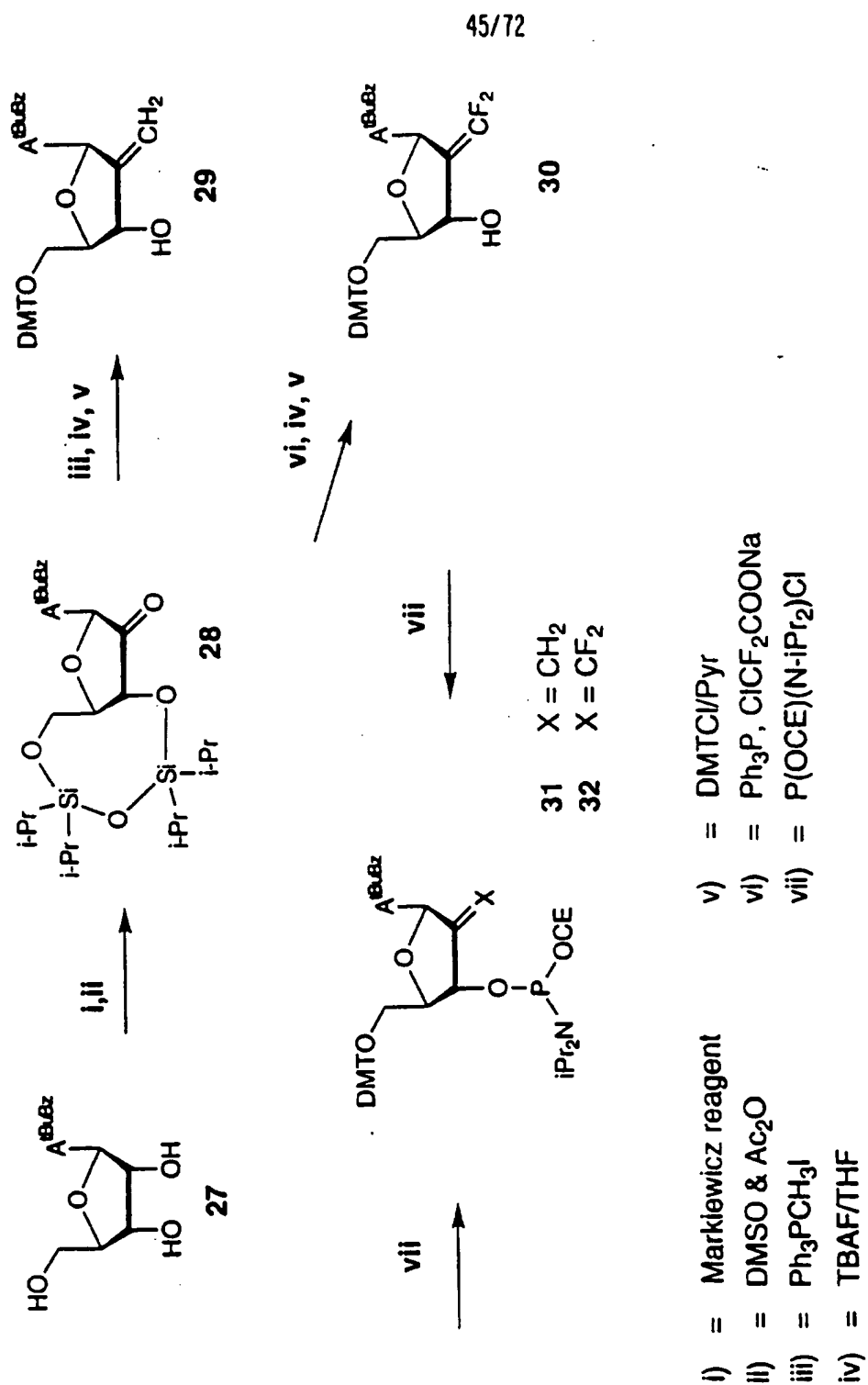
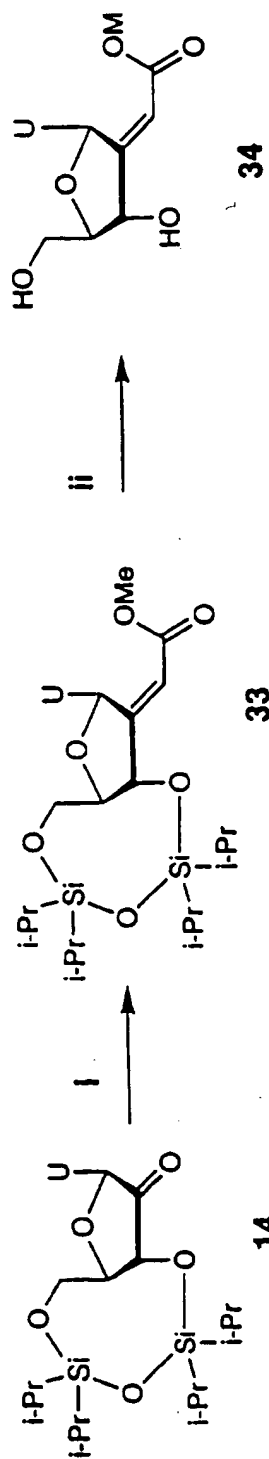


FIG. 52.



i) =  $\text{Ph}_3\text{PC}=\text{CHC}(\text{O})\text{OCH}_3\cdot\text{OAc}$

ii) =  $\text{NEt}_3\cdot 3 \text{ HF}$

iii) =  $\text{DMTCI/Pyr}$

iv) =  $\text{P}(\text{OCE})(\text{N-}i\text{Pr}_2)\text{Cl}$

v) =  $\text{MeOH/NaOH}$

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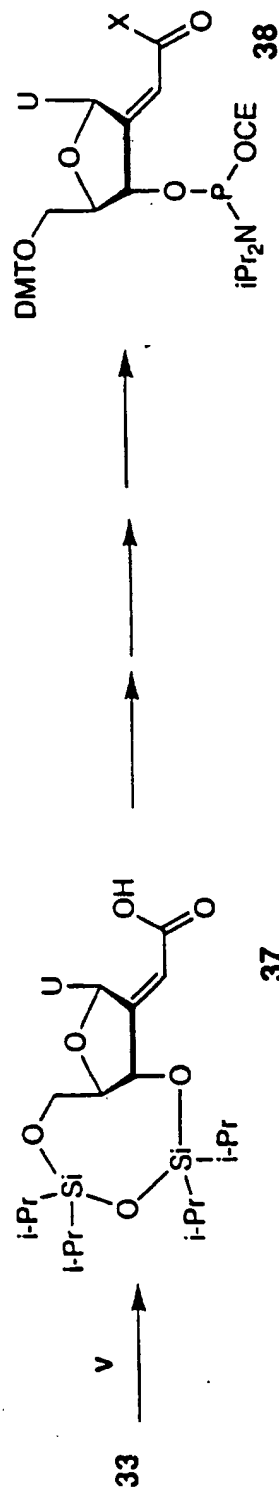
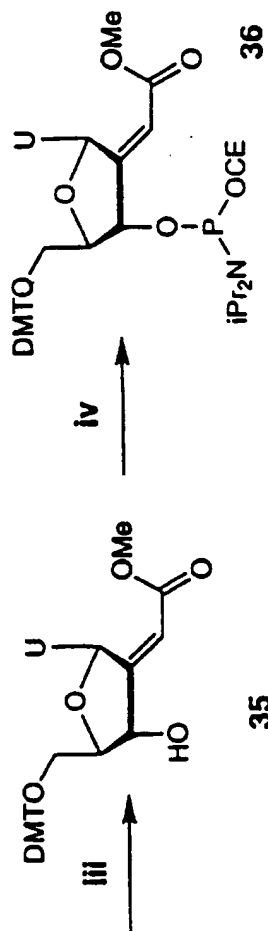
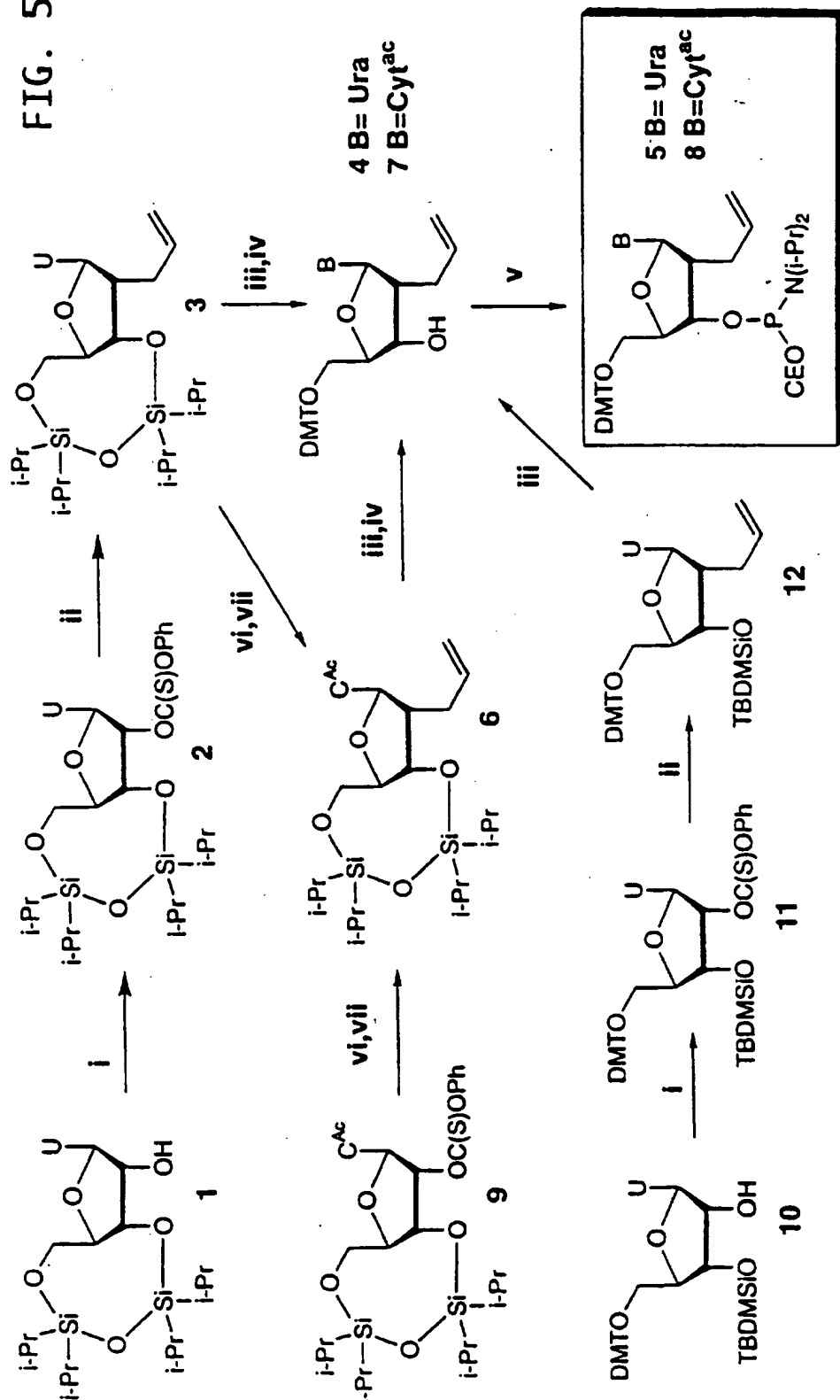


FIG. 53.



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FIG. 54.



i) = PhOC(S)Cl/Py ii) = Allyltributyltin, Bz<sub>2</sub>O<sub>2</sub> or AIBN /toluene/iii) = TBAF iv) = DMTCI/Py  
 v) = P(OCE)(N-iPr<sub>2</sub>)Cl vi) = 1,2,4-triazole, P(O)Cl<sub>3</sub> vii) = 29% NH<sub>4</sub>OH/dioxane, Ac<sub>2</sub>O/Py

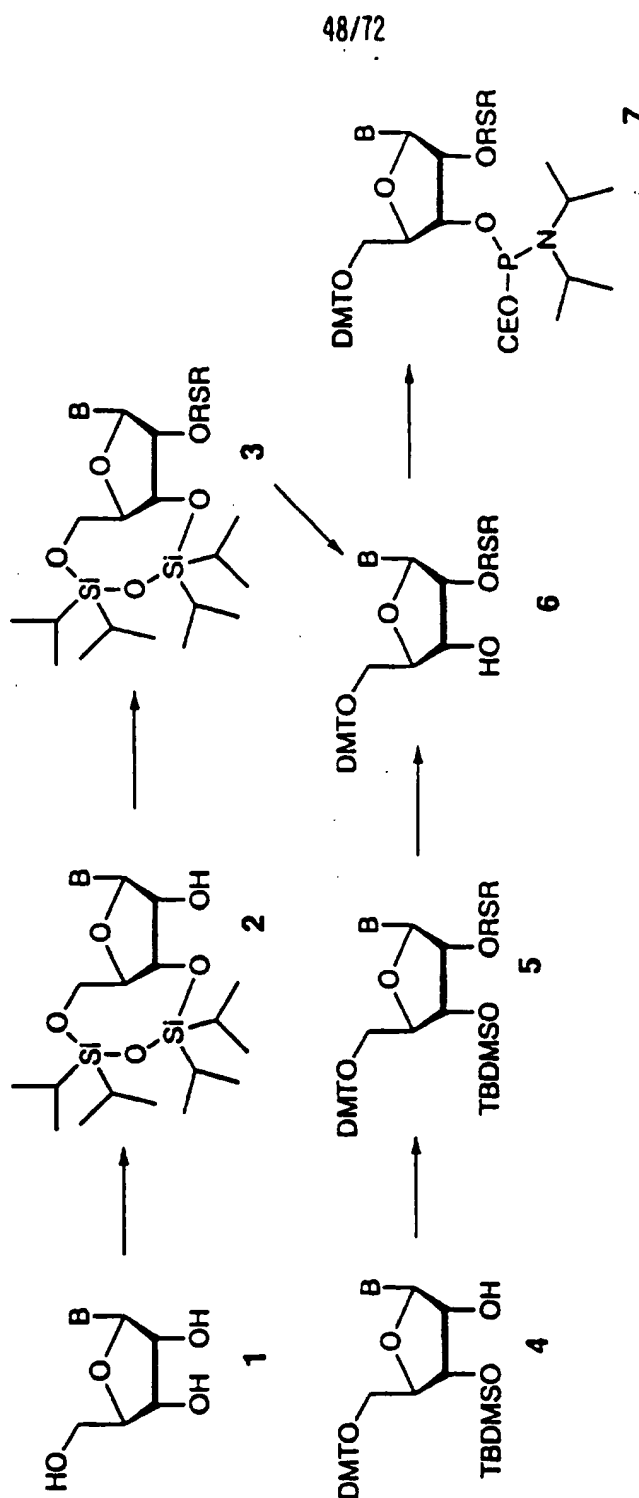


FIG. 55.

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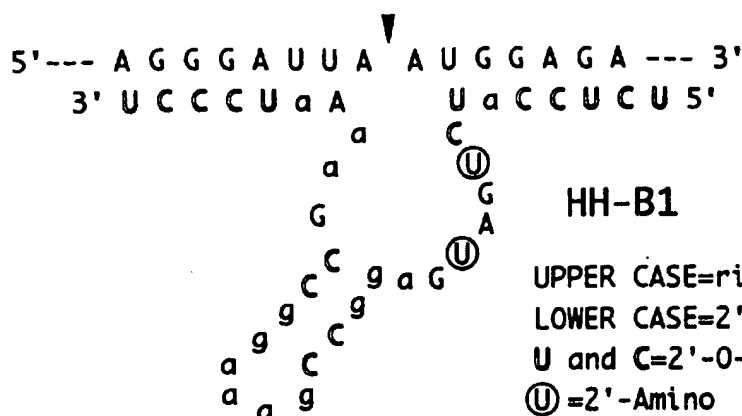


FIG. 56a.

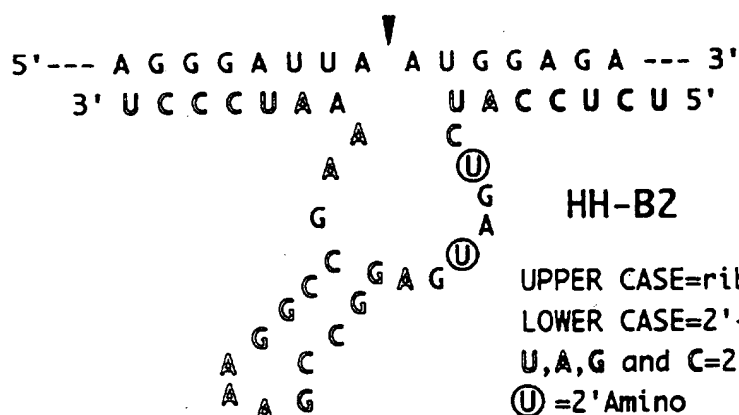


FIG. 56b.

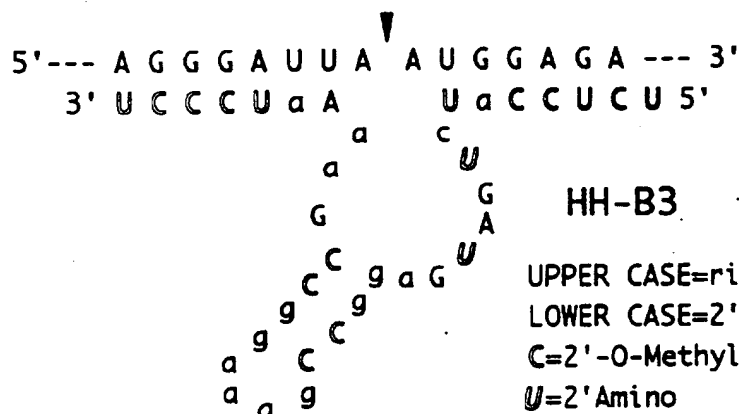


FIG. 56c.

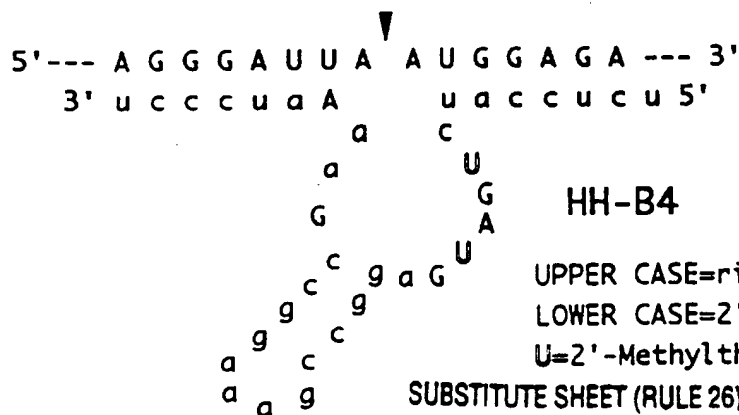


FIG. 56d.

SUBSTITUTE SHEET (RULE 26)

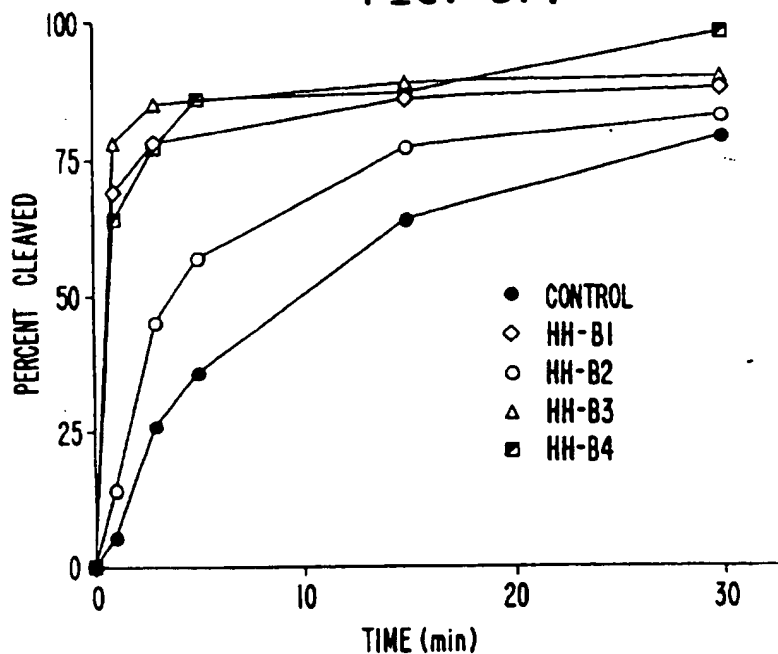
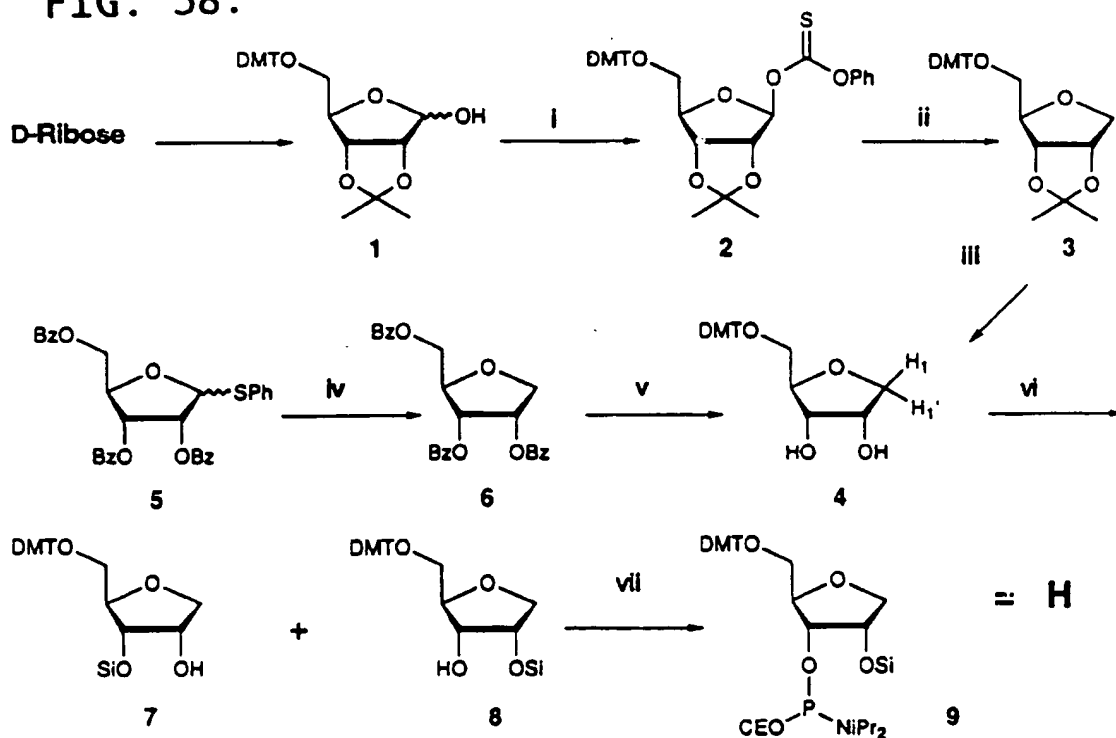
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FIG. 57.

FIG. 58.



Si = *t*-Butyldimethylsilyl  
 DMT = 4,4'-Dimethoxytrityl  
 CE = Cyanoethyl

Reagents and Conditions: i)  $\text{PhOC(S)-Cl/DMAP}$ , ii)  $\text{Bu}_3\text{SnH/AIBN}$ , iii)  $\text{CF}_3\text{COOH}$ , DMT-Cl/Pyr, iv)  $\text{Bu}_3\text{SnH/Bz}_2\text{O}_2$ , v)  $2\text{M NaOH/Pyr/MeOH}$ , DMT-Cl/Pyr, vi)  $\text{TBDMS-Cl/AgNO}_3$ , vii)  $\text{P(OCE)(N-iPr}_2\text{)Cl}$   
 SUBSTITUTE SHEET (RULE 26)

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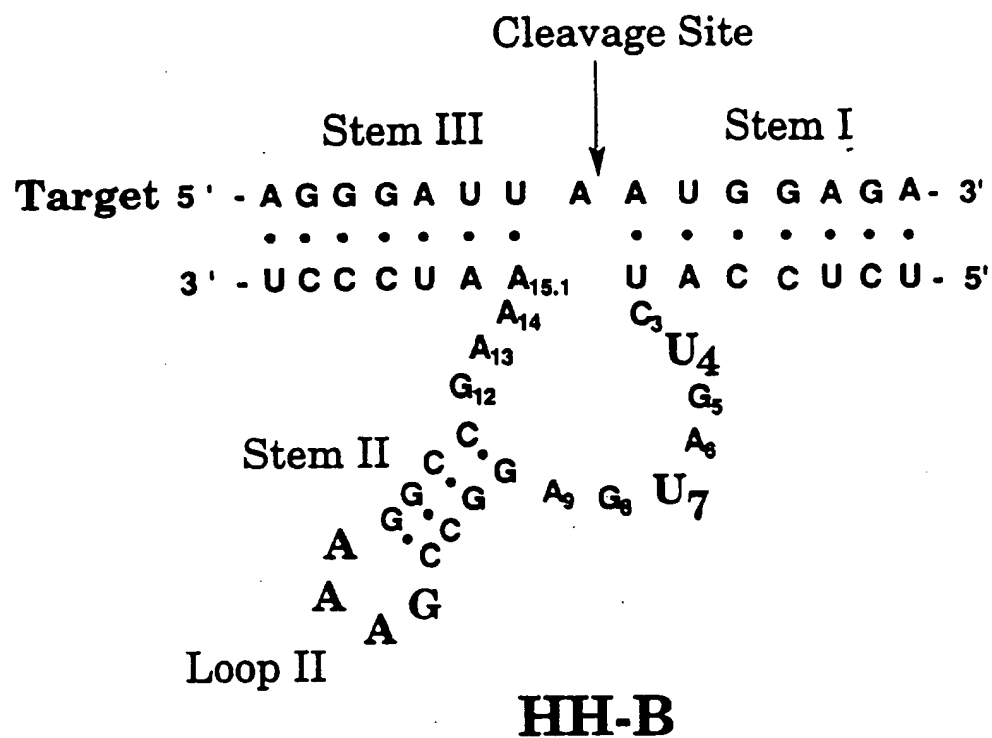


FIG. 59.

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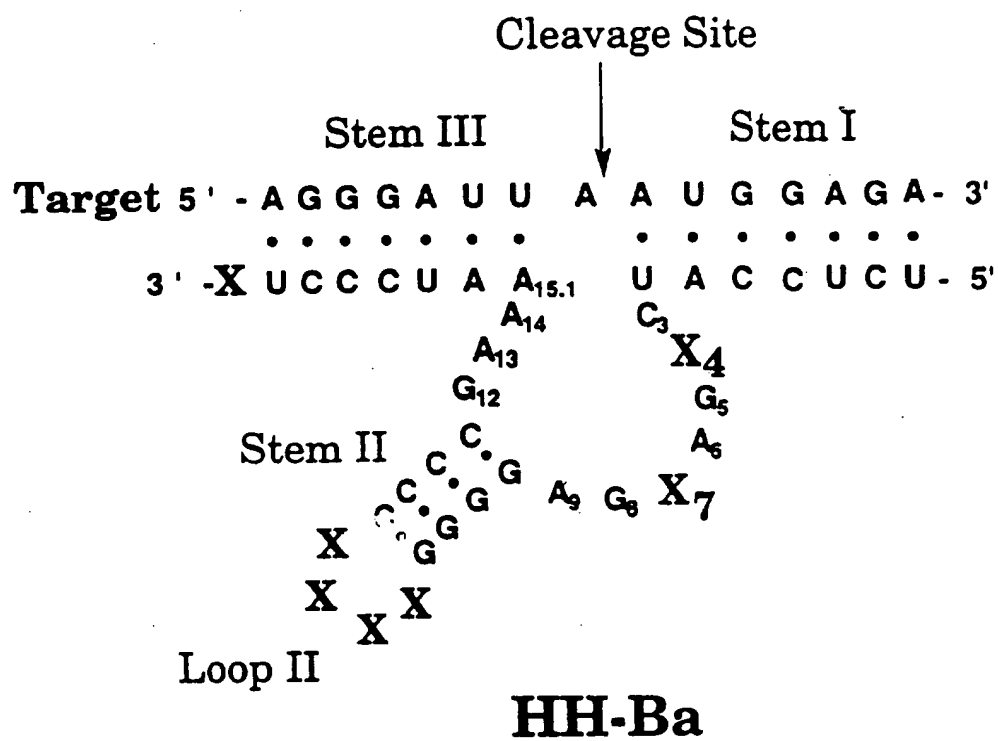


FIG. 60.

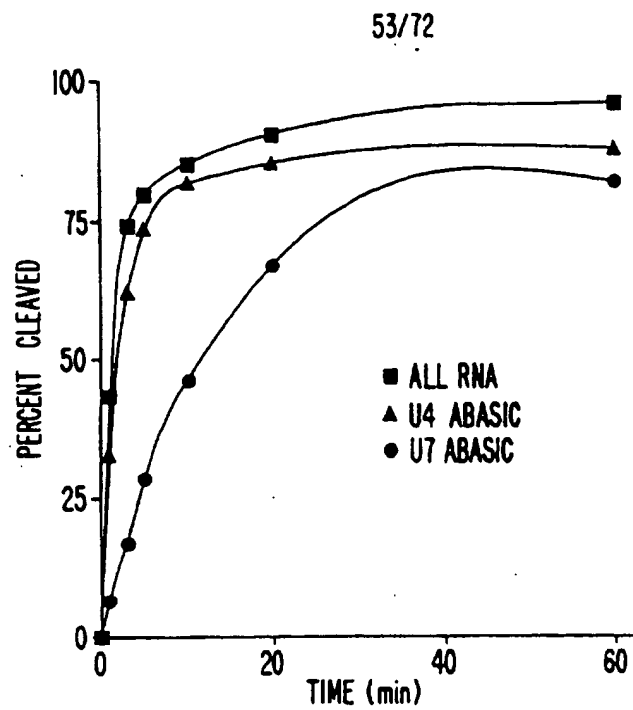


FIG. 61.

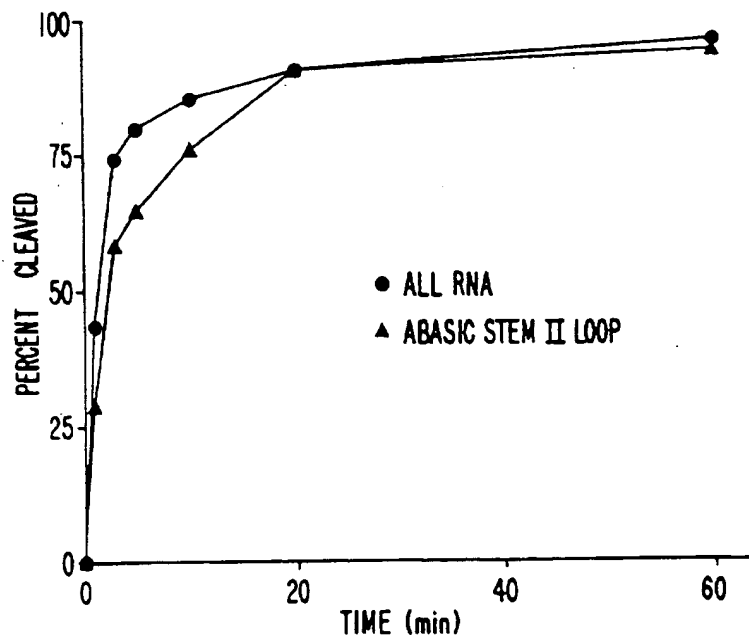


FIG. 62.

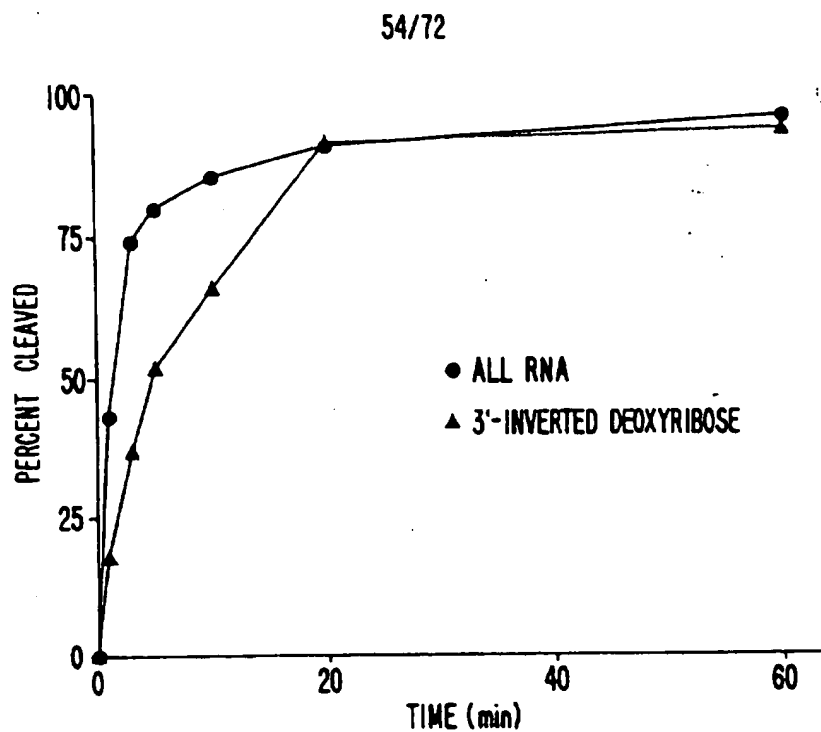


FIG. 63.

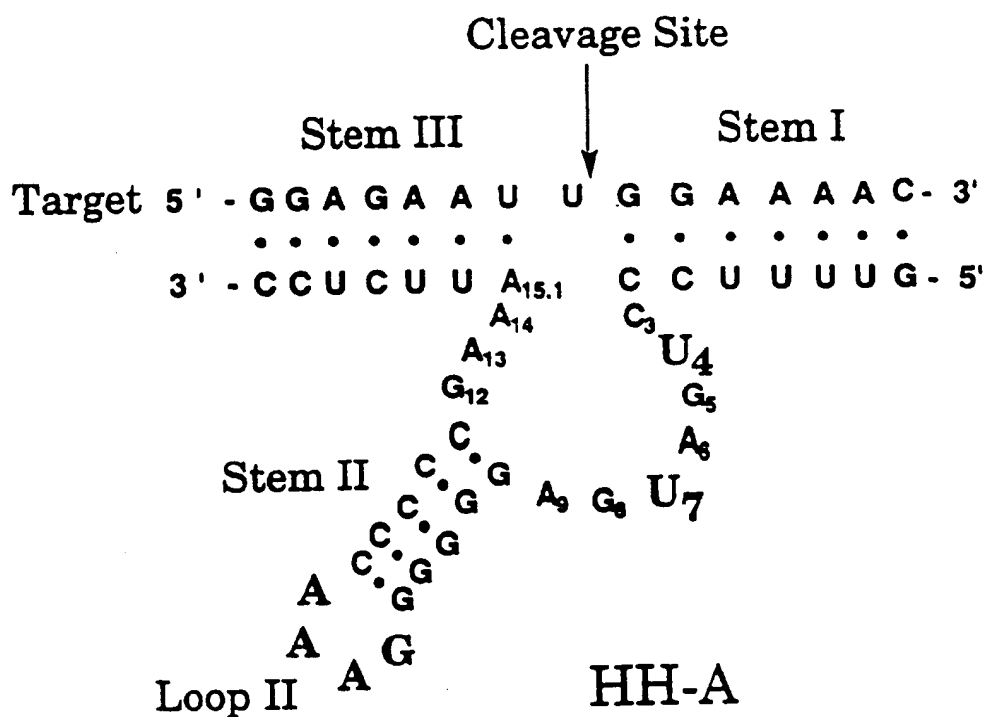


FIG. 64.

SUBSTITUTE SHEET (RULE 26)



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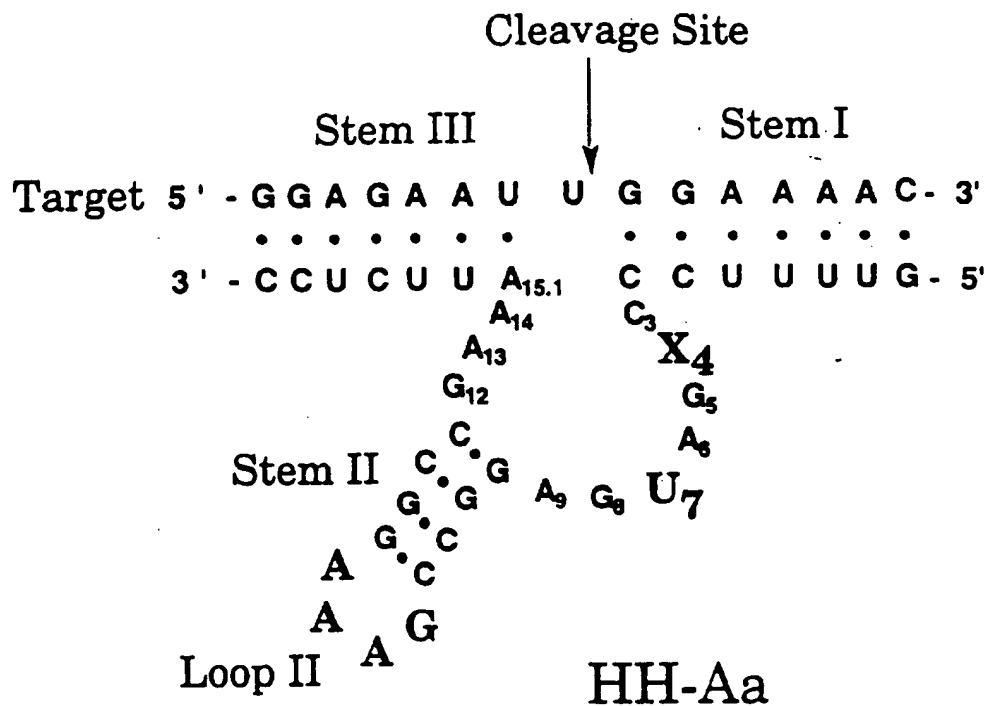


FIG. 65.

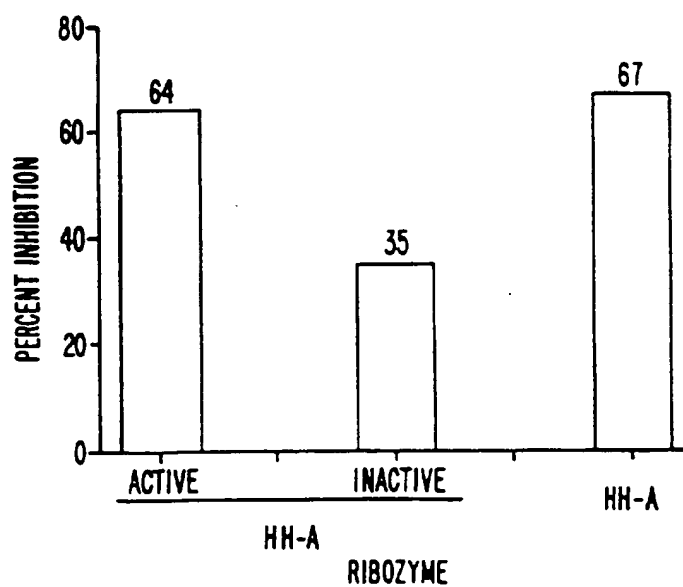


FIG. 66.

SUBSTITUTE SHEET (RULE 26)

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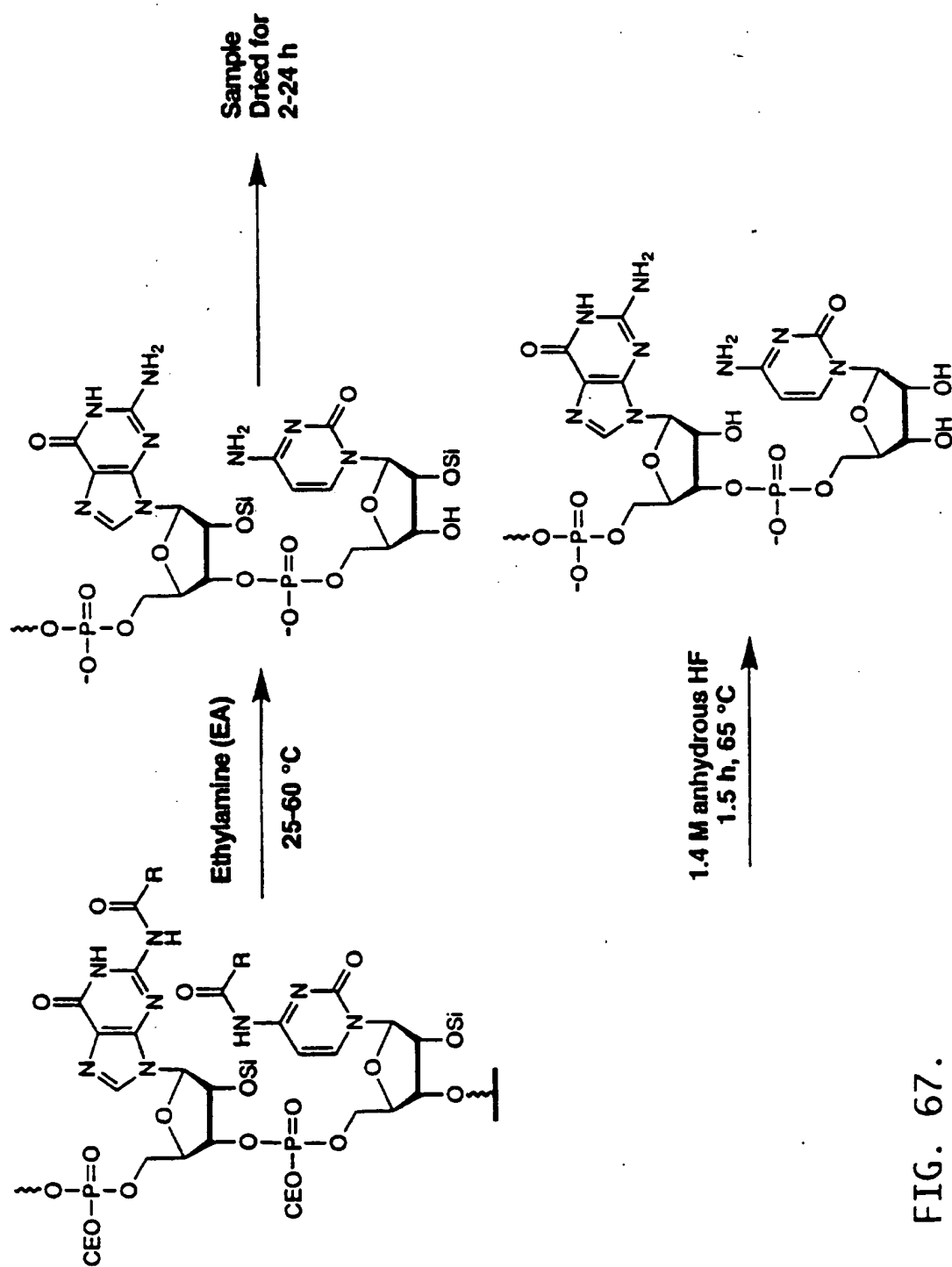
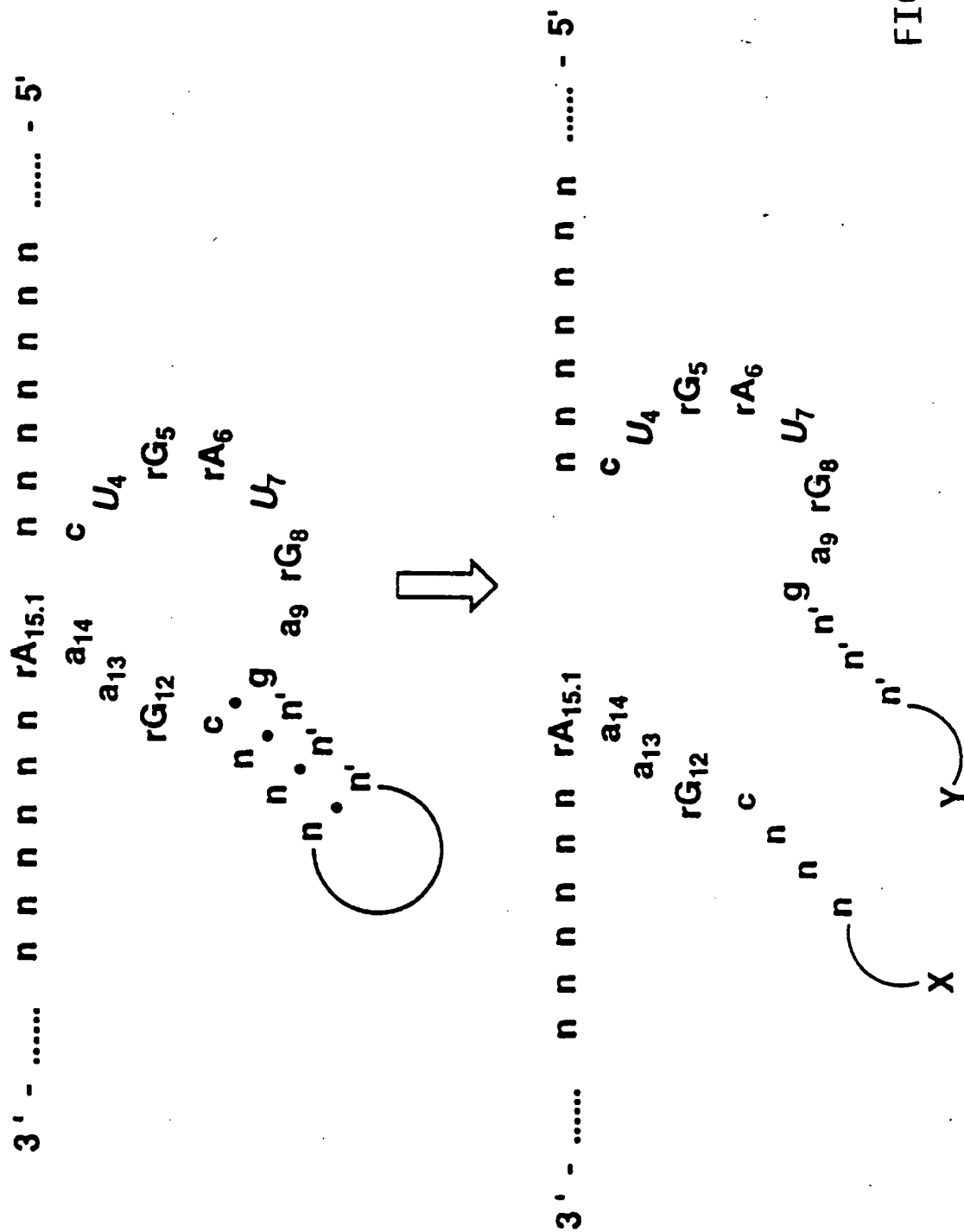
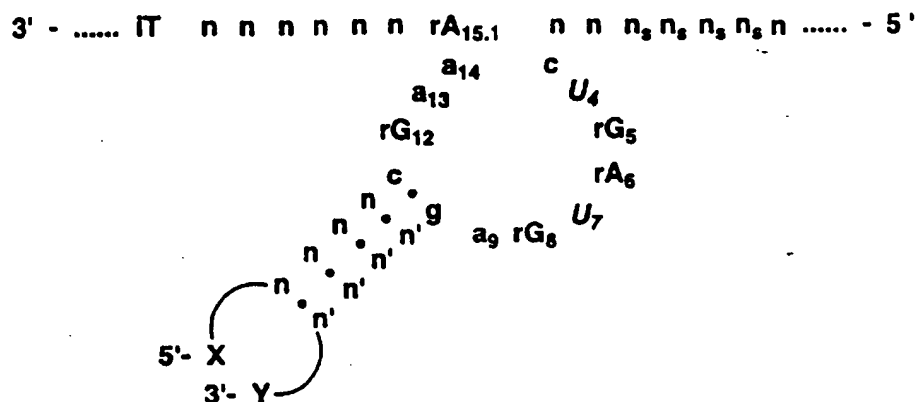


FIG. 67.



**FIG. 68.**

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NOTE: (CH<sub>2</sub>)<sub>n</sub> refers to any linkage. In addition, X and Y can be interchanged.

X = (CH<sub>2</sub>)<sub>n</sub>SH, Y = (CH<sub>2</sub>)<sub>n</sub>SH → disulfide

X = (CH<sub>2</sub>)<sub>n</sub>NHR, Y = ribose → morpholino

X = (CH<sub>2</sub>)<sub>n</sub>NHR, Y = CO<sub>2</sub>H → amide

X = (CH<sub>2</sub>)<sub>n</sub>X, Y = (CH<sub>2</sub>)<sub>n</sub>OH → ether, X = halogen

X = (CH<sub>2</sub>)<sub>n</sub>NHR, Y = CHO → amine

X = (CH<sub>2</sub>)<sub>n</sub>PPh<sub>3</sub>, Y = CHO → double bond

X = (CH<sub>2</sub>)<sub>n</sub>NHR, Y = (CH<sub>2</sub>)<sub>n</sub>SO<sub>2</sub>Cl → sulfonamide

X = (CH<sub>2</sub>)<sub>n</sub>OH, Y = CO<sub>2</sub>H → ester

X = (CH<sub>2</sub>)<sub>n</sub>X, Y = (CH<sub>2</sub>)<sub>n</sub>SH → thioether, X = halogen

X = (CH<sub>2</sub>)<sub>n</sub>COX, Y = (CH<sub>2</sub>)<sub>n</sub>OH → carbonate, X = halogen

FIG. 69.

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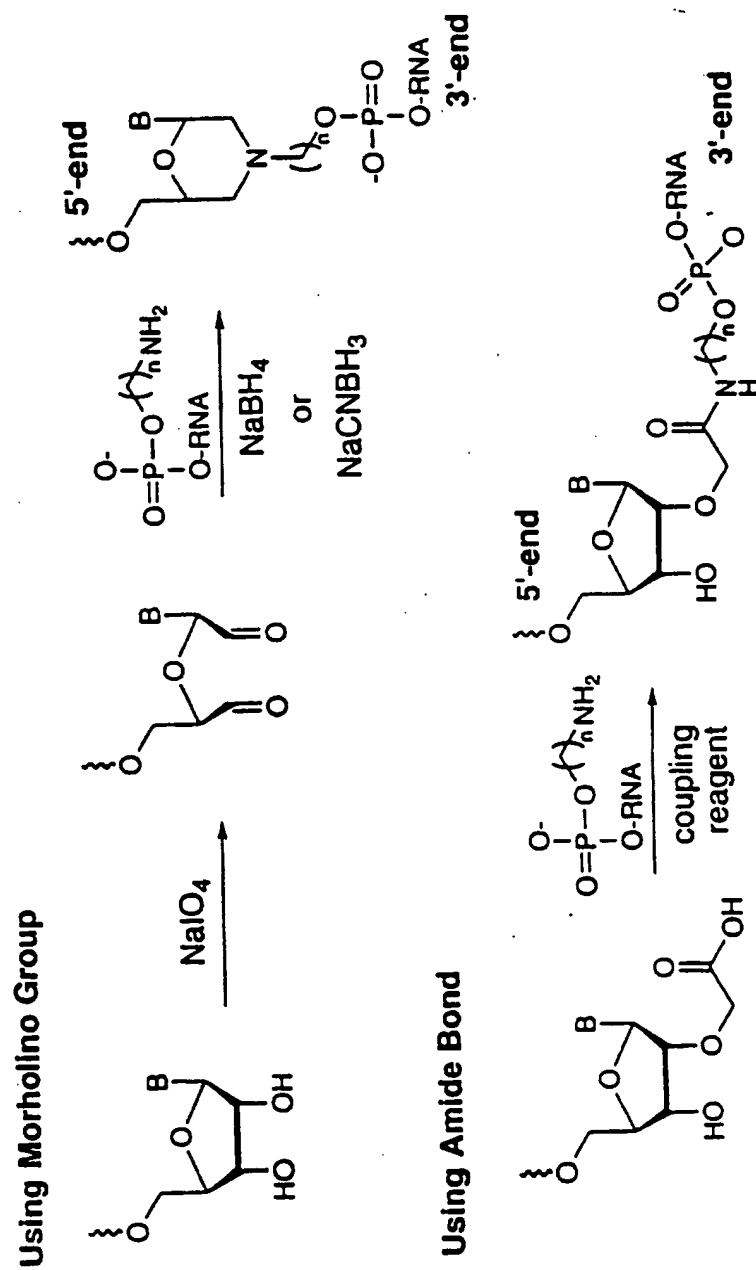
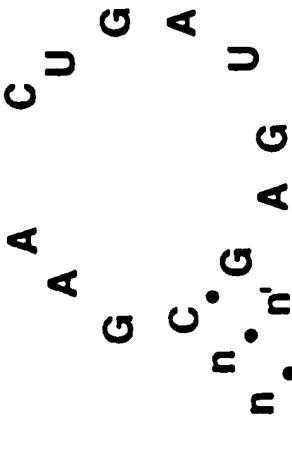


FIG. 70.

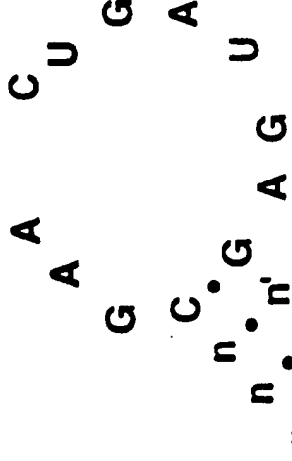
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3' - ..... CCUCUUA CCUUUUG ..... - 5'



HH-A1

3' - ..... CCUCUUA CCUUUUG ..... - 5'



HH-A2; x = 5  
HH-A3; x = 6

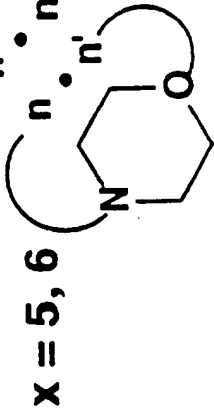


FIG. 71.

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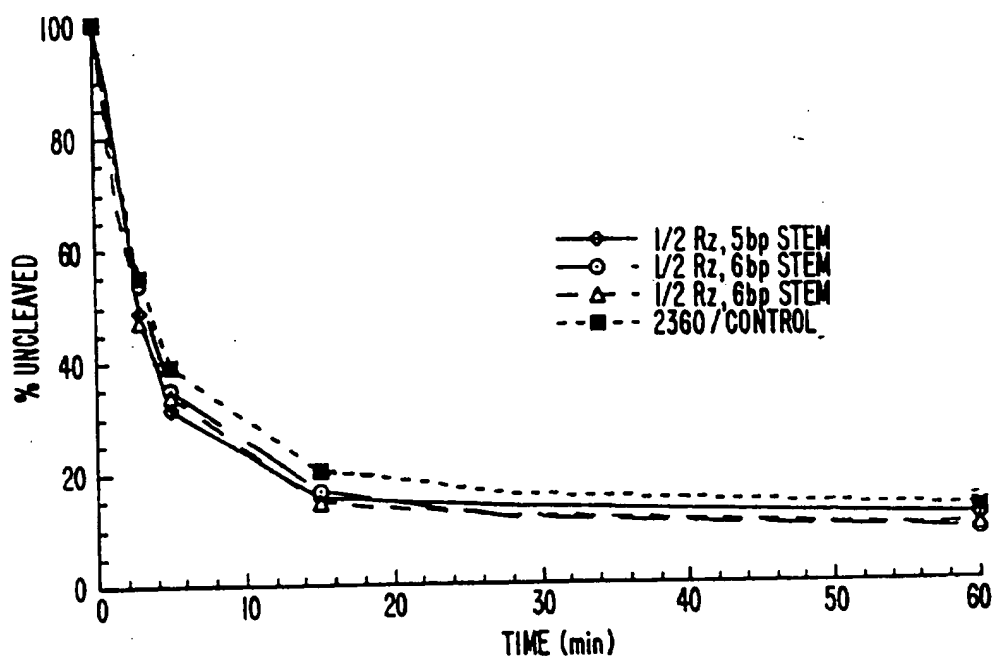


FIG. 72.

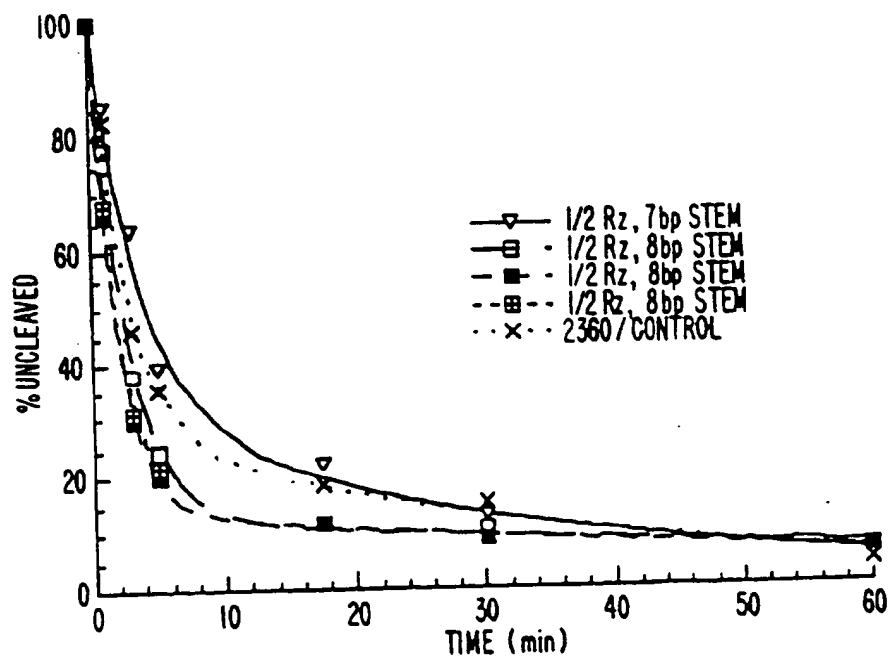


FIG. 73.

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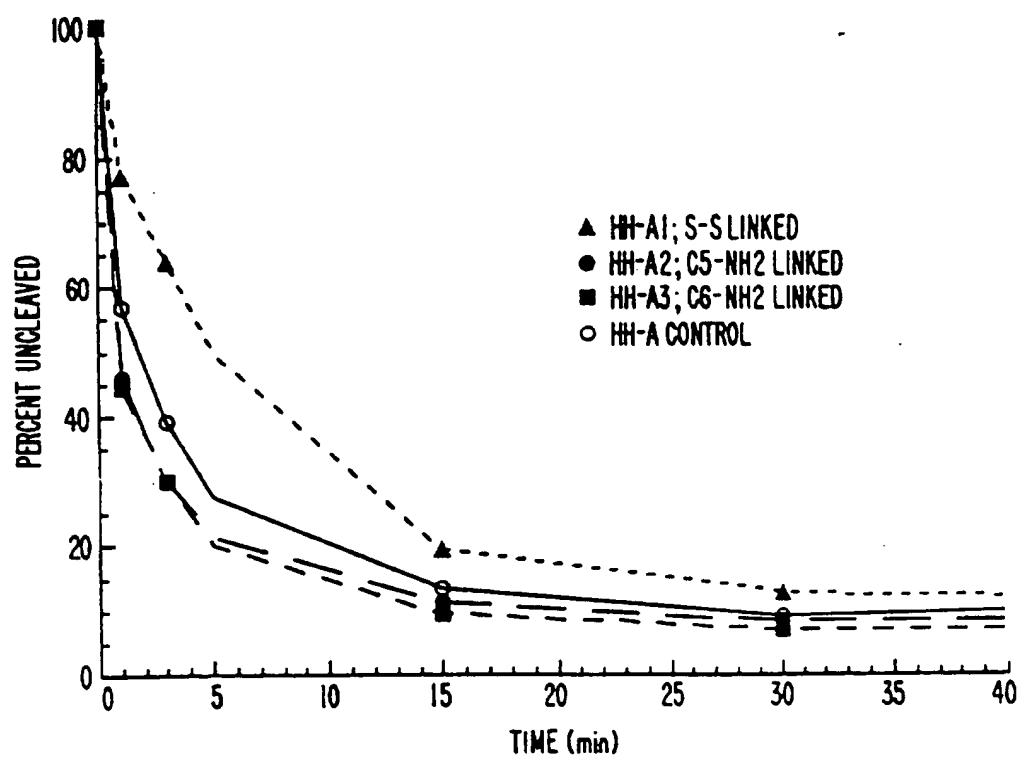
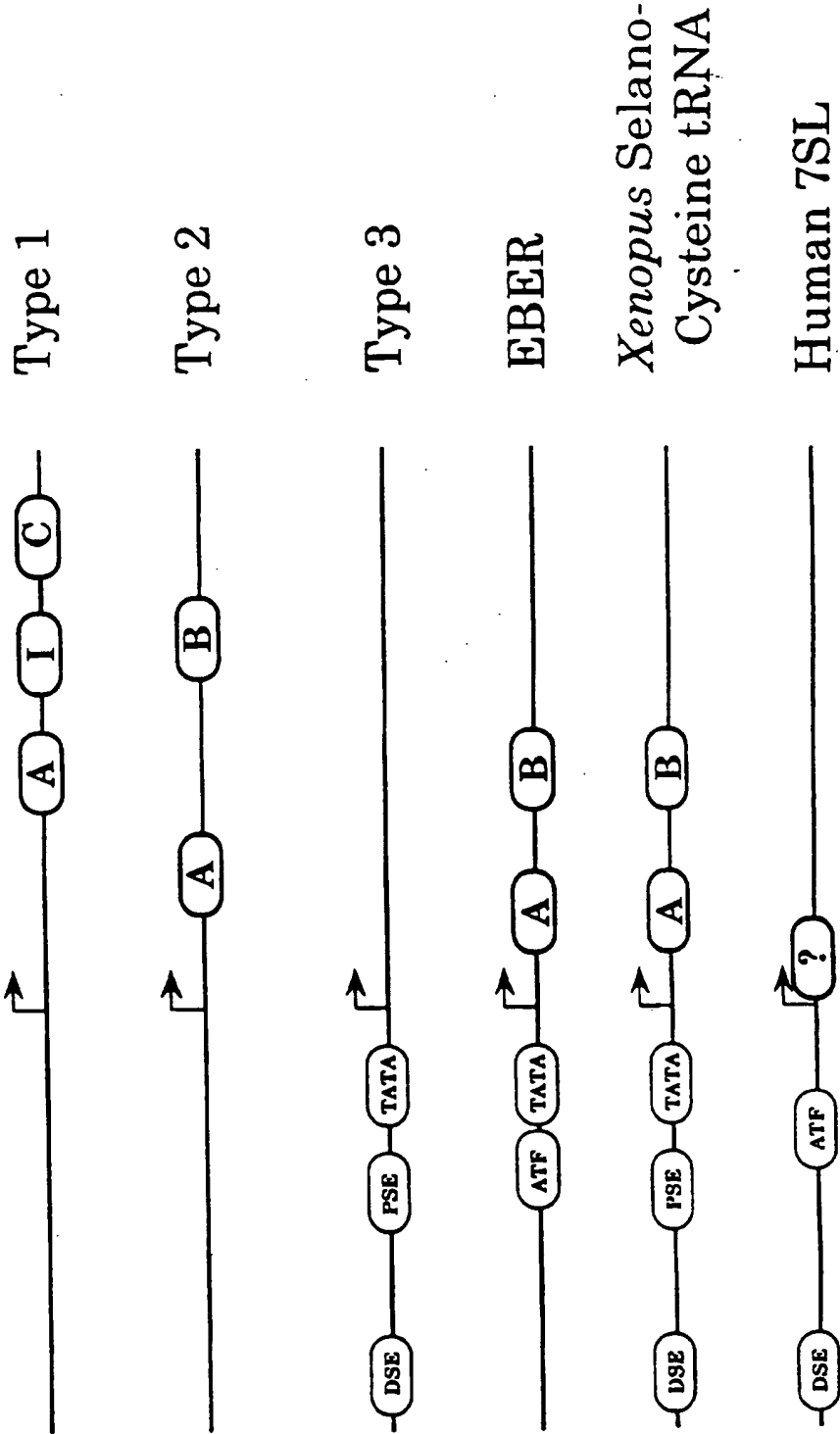


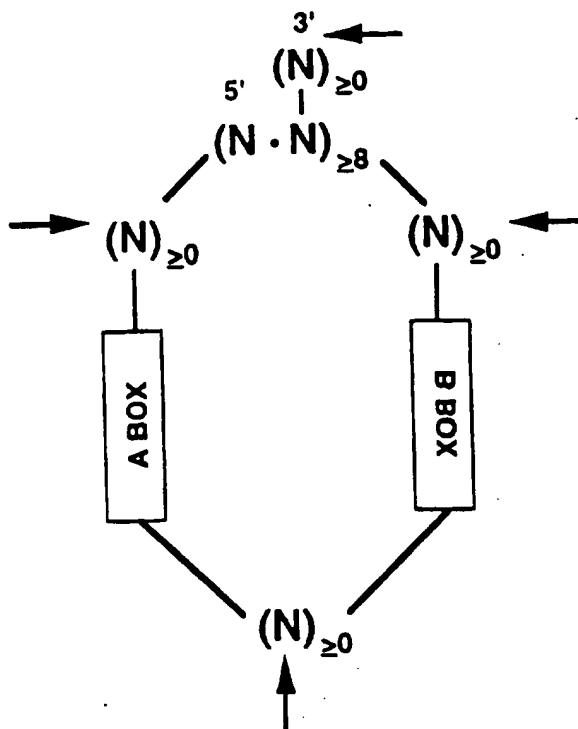
FIG. 74.



FIG. 75.



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A BOX = URGCNNAGYGG

B BOX = GGUUCGANUCC

This is based on Geiduschek & Tocchini-Valentini, (1988) Annu. Review Biochem. 57, 873-914. However this consensus sequence is not meant to be limiting

N = A, U, G, or C

R = Purine

Y = Pyrimidine

• = Indicates base-pairing

— = Indicates covalent linkage

➔ = Indicates sites at which desired RNAs can be cloned

FIG. 76.

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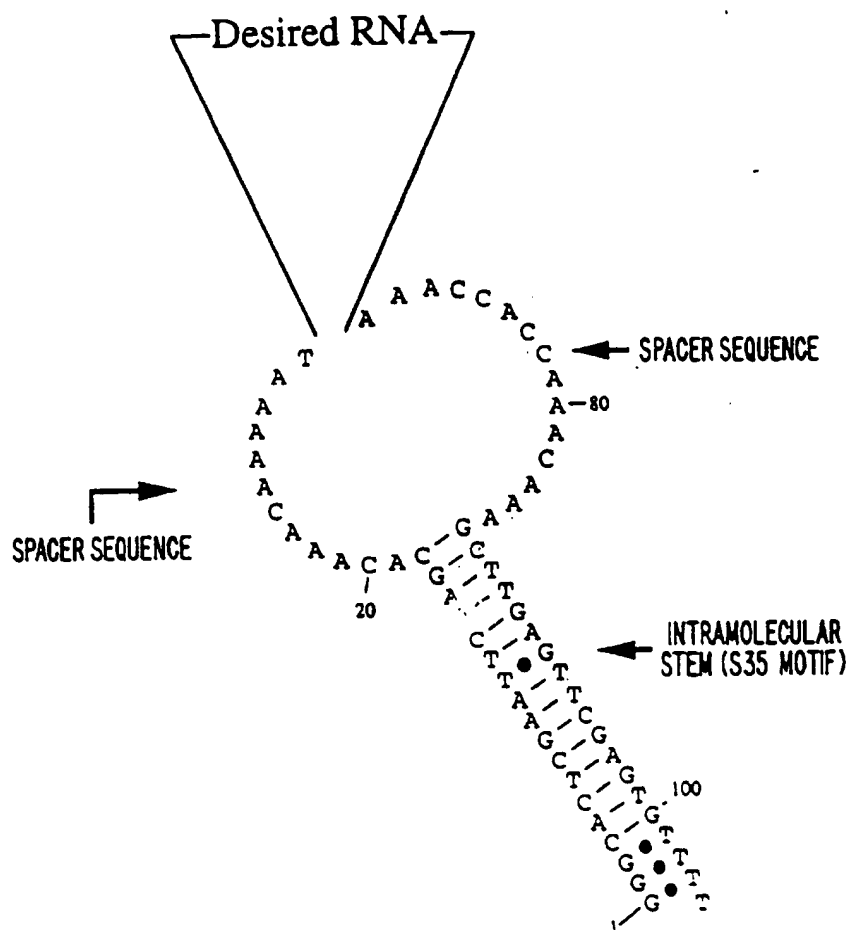


FIG. 77.





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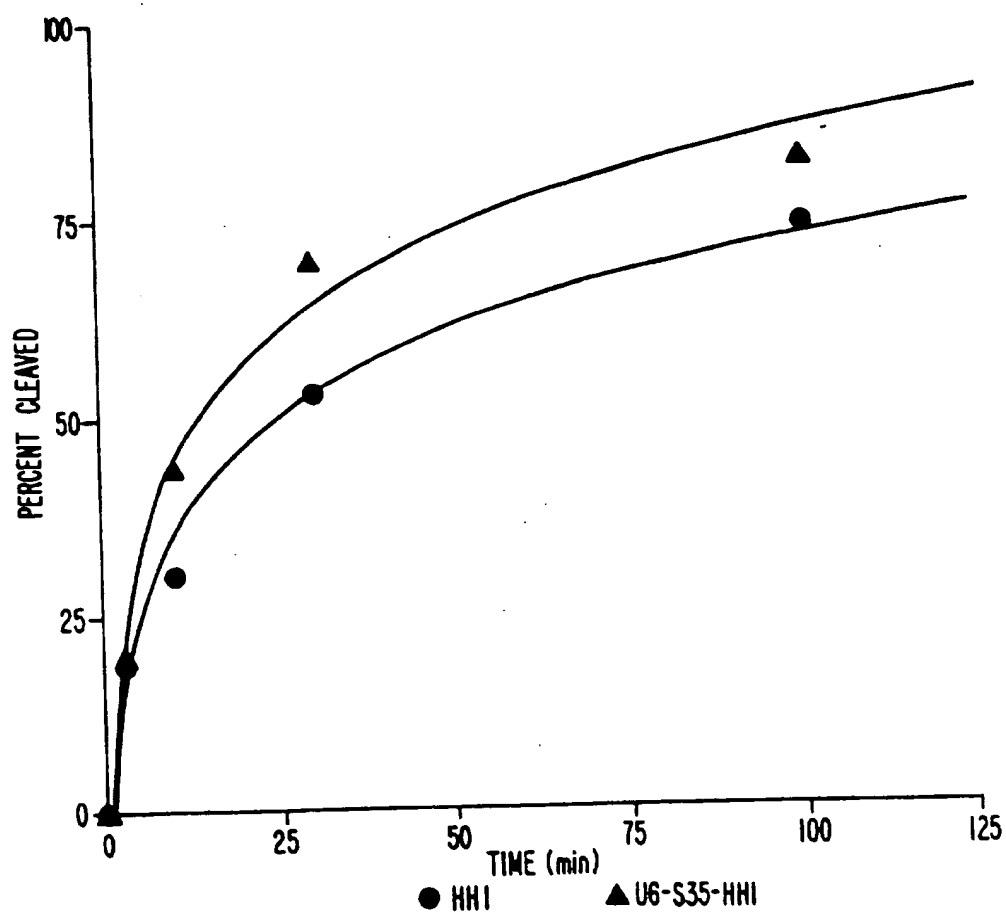


FIG. 80.

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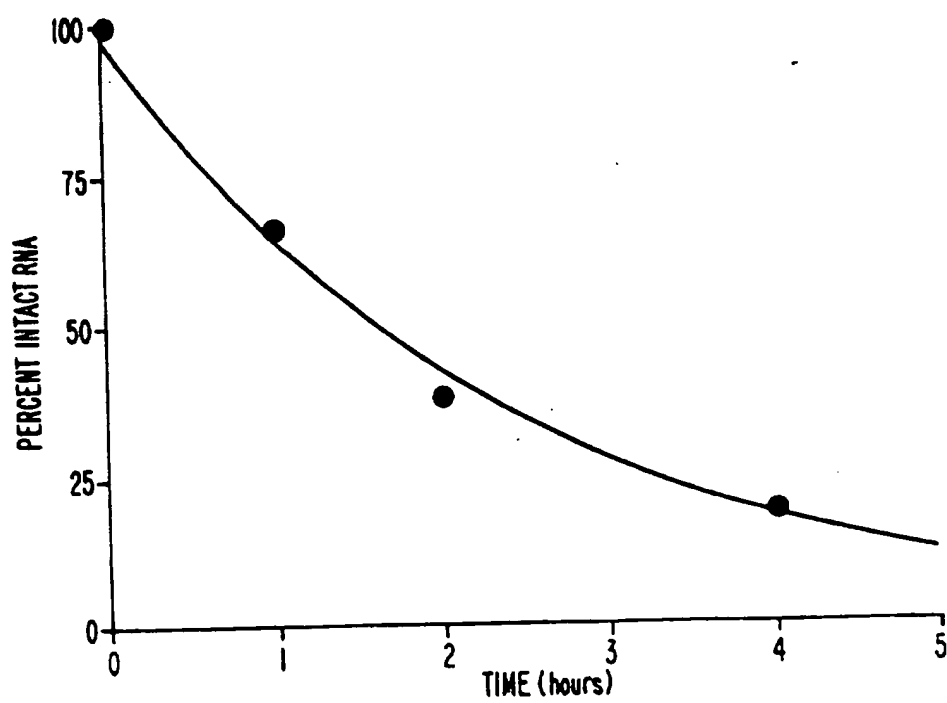
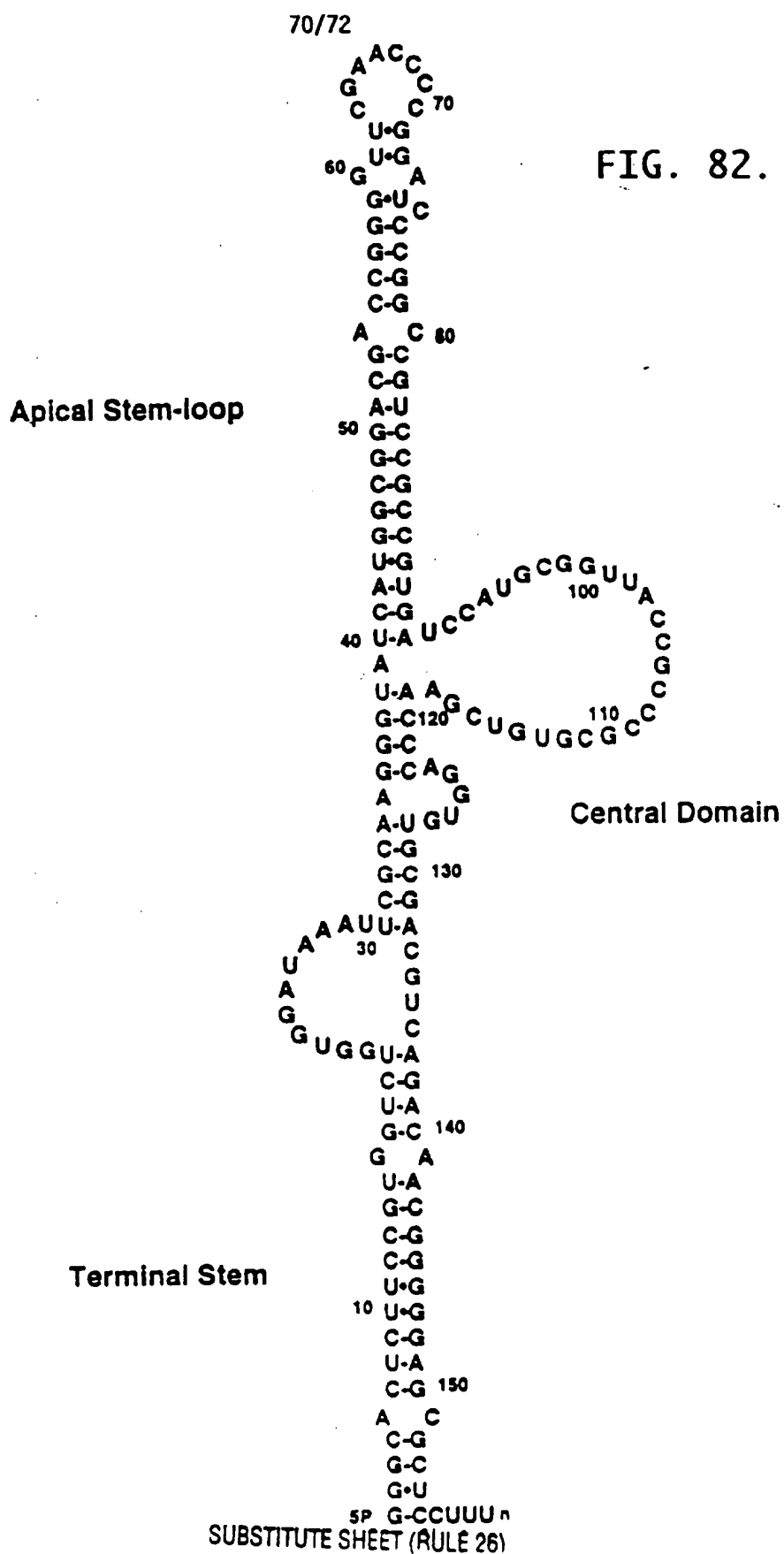
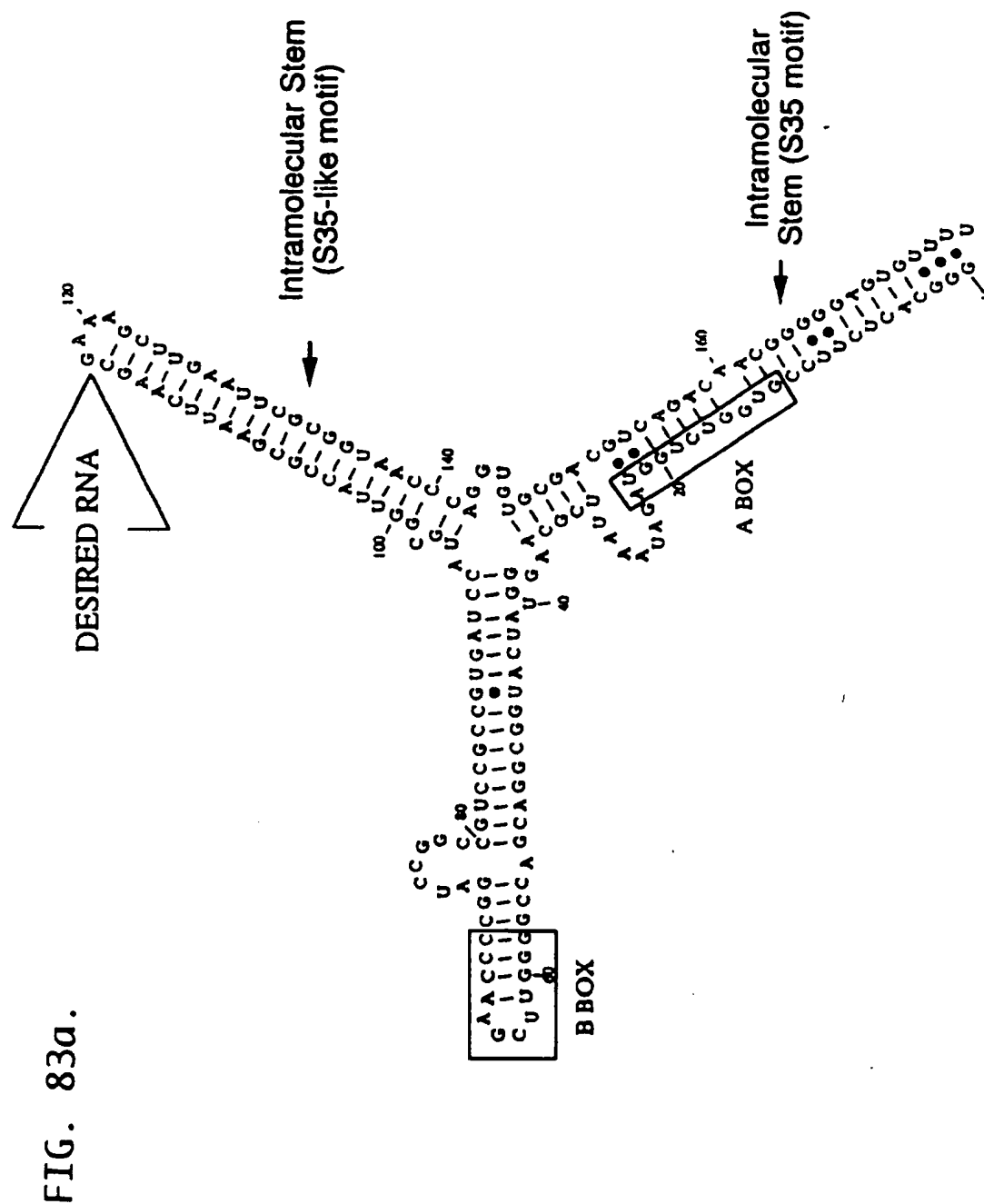


FIG. 81.







## VA1-Chimera

# HHI

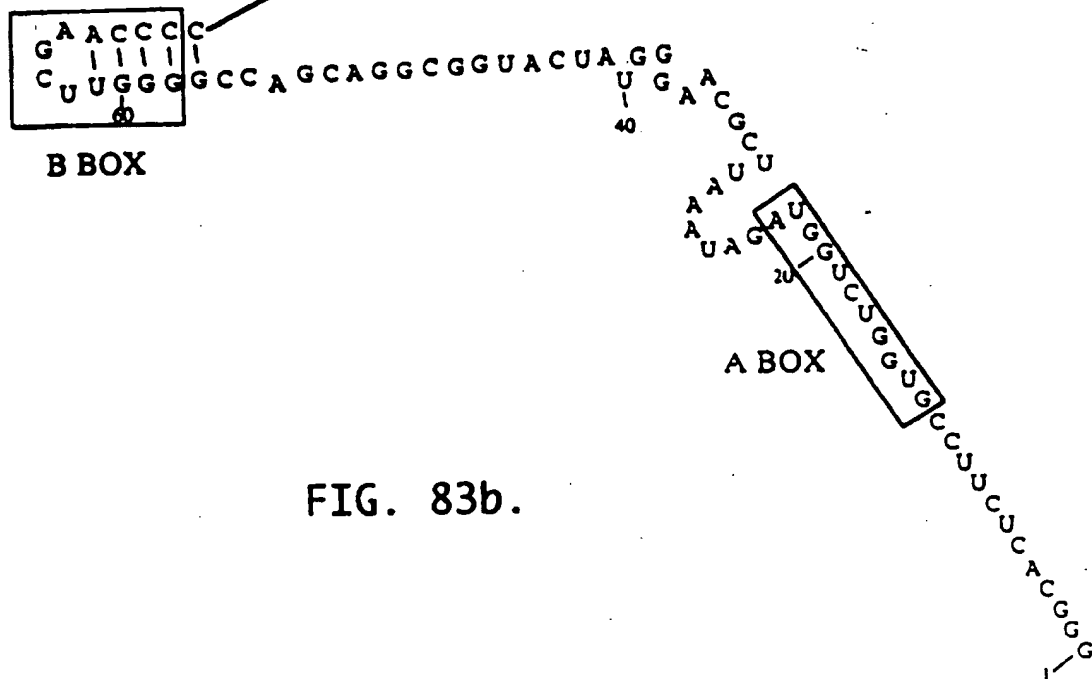
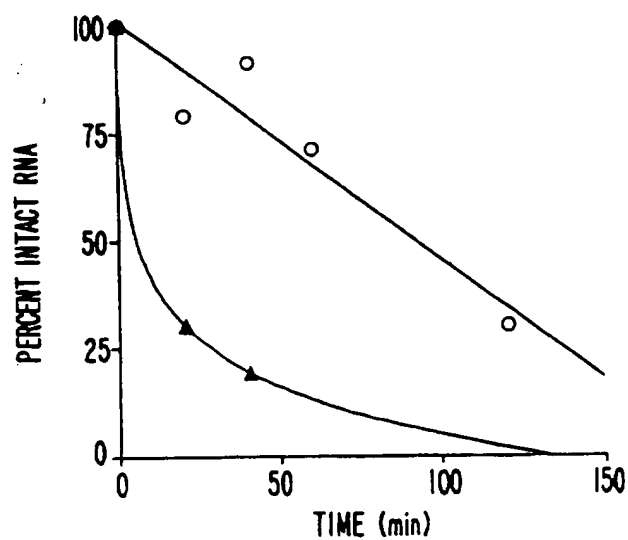


FIG. 83b.



▲ VAI-CHIMERA

○ VAI-S35-CHIMERA

FIG. 84.